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SEROTONIN RECEPTORS AND THE ACTIONS OF TWO PHENETHYLAMINE HALLUCINOGENS ON THE FIRING OF SINGLE CELLS IN THE RAT CENTRAL NERVOUS SYSTEM

BY

NICHOLAS J. PENINGTON

A THESIS

SUBMITTED TO THE FACULTY OF GRADUATE STUDIES AND RESEARCH IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF DOCTOR OF PHILOSOPHY

DEPARTMENT OF PHARMACOLOGY

EDMONTON, ALBERTA

SPRING, 1986

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"But, keeping still the end in view
to which I hoped to come,
I strove to prove the matter true
by putting everything I knew
into an axiom"

Lewis Carroll

Phantasmagoria

1869

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FACULTY OF GRADUATE STUDIES AND RESEARCH

The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies and Research, for acceptance, a thesis entitled "Serotonin receptors and the actions of two phenethylamine hallucinogens on the firing of single cells in the rat central nervous system" submitted by Nicholas J. Penington in partial fulfillment of the requirements of the degree of Doctor of Philosophy in Pharmacology.

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External Examiner

Date 14th Feb 1986.

ABSTRACT

The potent hallucinogenic phenethylamine 2,5-dimethoxy-4-methyl-amphetamine (DOM or "STP") is one of several amphetamine derivatives which can be differentiated both behaviourally and neurochemically from the indirectly acting compound D-amphetamine. DOM has been proposed to be an agonist at specific neuronal receptors in the central nervous system which recognize the putative neurotransmitter substance serotonin (5-HT). To date no direct test of this proposal has been made.

In this thesis electrophysiological techniques were employed to record extracellularly the activity of single neurones in the brain of anaesthetized rats. The responses of single cells to direct iontophoretic application of the putative neurotransmitters and hallucinogens were studied. The effects of these compounds were observed primarily on cells with a serotonergic input such as hippocampal CA_1 pyramidal neurones or facial motor neurones (FMN's) and also putative 5-HT-containing cells of the dorsal raphé (DR) nucleus. The responses of cells to 5-HT in these three areas are reportedly mediated by different receptor subtypes.

Results in this thesis show that DOM could produce a maximal inhibition of hippocampal CA_1 cell firing rate, but was not as effective in terms of ejection current as was 5-HT. All 5-HT antagonists were ineffective at preventing 5-HT-induced inhibition of hippocampal CA_1 cells and so the 5-HT receptor dependency of the

effects of DOM could not be determined. An examination of the effect of DOM applied directly to DR neurones suggested a difference between the phenethylamine DOM, on the one hand, and the indolealkylamines, LSD and D-amphetamine on the other. DOM was not a potent inhibitor of presumed serotonergic DR neurones, and unlike 5-HT, LSD and D-amphetamine, was not capable of producing more than a 20% reduction in DR cell firing rate. Thus indolealkylamine hallucinogens and LSD may be more potent inhibitory agonists on DR presynaptic receptors (S_2) than at their projection sites, but this appears not to be the case for the phenethylamine group.

Behavioural, binding, and classical pharmacological studies indicate that DOM may be a "D" (or 5-HT_2 receptor) agonist; 5-HT_2 type receptors may mediate 5-HT-induced excitation in the central nervous system. Results of this thesis demonstrate that DOM excited FMN's in a similar fashion to 5-HT. In contrast to its effects on 5-HT-induced inhibition of CA_1 pyramidal cells, methysergide was a selective antagonist of 5-HT- and of DOM- induced excitations of FMN's. It was also discovered that dopamine (DA) facilitated the firing of FMN's, probably by an interaction with its own specific receptors. This conclusion was reached after it was shown that the neuroleptic chlorpromazine selectively antagonized the responses of FMN's to DA. Unfortunately, a local anaesthetic effect of chlorpromazine during application of DOM prevented the ruling out of a mixed agonist effect of DOM on receptors for both 5-HT and DA.

Ketanserin, a 5-HT_2 receptor-selective antagonist, also antagonized the action of 5-HT on these neurones. This supports the proposal that an interaction with 5-HT_2 receptors may underlie or contribute to the mechanism of action of hallucinogenic drugs.

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LIST OF ABBREVIATIONS

ACh.....acetylcholine

DA.....dopamine

5,7-DHT.....5,7-dihydroxytryptamine

2,5-DMA.....2,5-dimethoxyamphetamine

DOB.....2,5-dimethoxy-4-bromoamphetamine

DOET.....2,5-dimethoxy-4-ethylamphetamine

DOI.....2,5-dimethoxy-4-iodoamphetamine

DOM.....2,5-dimethoxy-4-methylamphetamine

DR.....dorsal raphé

E.P.S.P.....excitatory post-synaptic potential

FMN.....facial motor neurone

GABA.....γ-aminobutyric acid

GLU.....glutamate

5-HT.....5-hydroxytryptamine

5-HTP.....5-hydroxytryptophan

I.P.S.P.....inhibitory post-synaptic potential

LGN.....lateral geniculate neurones

L-dopa.....L-3,4-dihydroxyphenylalanine

LSD.....lysergic acid diethylamide

m.....metre

MΩ.....megaohm

MAOI.....monoamine oxidase inhibitor

LIST OF ABBREVIATIONS

MDA.....methylenedioxyamphetamine

5-MeoDMT.....5-methoxy-N,N-dimethyltryptamine

5-MeoT.....5-methoxytryptamine

min.....minute

V.....volt

nA.....nanoampere

NA.....noradrenaline

PCPA.....para-chlorophenylalanine

PMA.....para-methoxyamphetamine

sec.....second

SPS.....spikes per second

CHAPTER I

INTRODUCTION

A. General Introduction

1. The problem

In 1940 Erspamer isolated from the salivary gland of the octopus an amine which he named enteramine. This compound was shown to be a powerful activator of some intestinal smooth muscles. Later, in 1948 Rapport et al. (1948) isolated from blood serum a vasoconstrictor substance which they named serotonin. Its structure was elucidated as that of 5-hydroxytryptamine (5-HT). In 1952 Erspamer and Asero (1952) identified enteramine as serotonin (5-HT); only a year later Twarog and Page (1953) found 5-HT in mammalian brain. Since that time a great deal of evidence has been amassed which suggests that 5-HT is a central neurotransmitter (Krnjevic, 1974). However the lack of specific antagonists has hindered proof of this hypothesis. Despite this difficulty, almost from the start of scientific work on 5-HT, there has been an association suspected between the central actions of 5-HT and hallucinogenic molecules. At first this was based upon their structural similarities, and later on pharmacological interactions (Gaddum, 1954).

2,5-Dimethoxy-4-methylamphetamine (DOM) is a hallucinogen which has LSD-like effects and is one-tenth as potent by the oral route as LSD (Shulgin, 1975). Whilst LSD and hallucinogens containing the indole structure have a range of potent and specific actions on central tryptaminergic neurones (first suggested by Gaddum in 1954), there is little or conflicting evidence about the actions of hallucinogenic amphetamines (see Section G.3 of Introduction). The

mechanism of action of this group of compounds has been differentiated into indirectly-acting amphetamine-like compounds (*para*-methoxyamphetamine (PMA), 3,4-dimethoxyamphetamine (3,4-DMA), and methylenedioxyamphetamine (MDA)) (PMA, 3,4-DMA, and MDA), and those that probably have direct agonist actions on brain 5-HT receptors (2,5-dimethoxy-4-methylamphetamine (DOM), 2,5-dimethoxy-4-bromoamphetamine (DOB) and 2,5-dimethoxy-4-ethylamphetamine (DOET, see Fig. 1). At least two reviews have concluded that more information on the central interaction between phenethylamine hallucinogens and the brain serotonergic system is required before we can assess its role in hallucinogenesis (Brawley and Duffield, 1971; Nichols and Glennon, 1984).

> 'Electrophysiologic studies have also failed to identify an anatomic or physiologic basis for the action of phenethylamine-type hallucinogens. Although it is generally believed that the phenethylamines act through a serotonergic mechanism, and this is supported by neurochemical and *in vitro* studies, their locus of action remains obscure' (Nichols and Glennon, 1984).

DOM was chosen as the prototype phenethylamine for this study because of its high potency and its proposed direct agonist of action on central 5-HT receptors (Wallach et al., 1972; Andén et al., 1974; Cheng et al., 1974a, 1974b; Nichols et al., 1982). It was decided to compare the effects of DOM on central 5-HT receptors which had been previously studied by microiontophoretic techniques (Aghajanian, 1981). This method involves the direct application of drugs, coupled

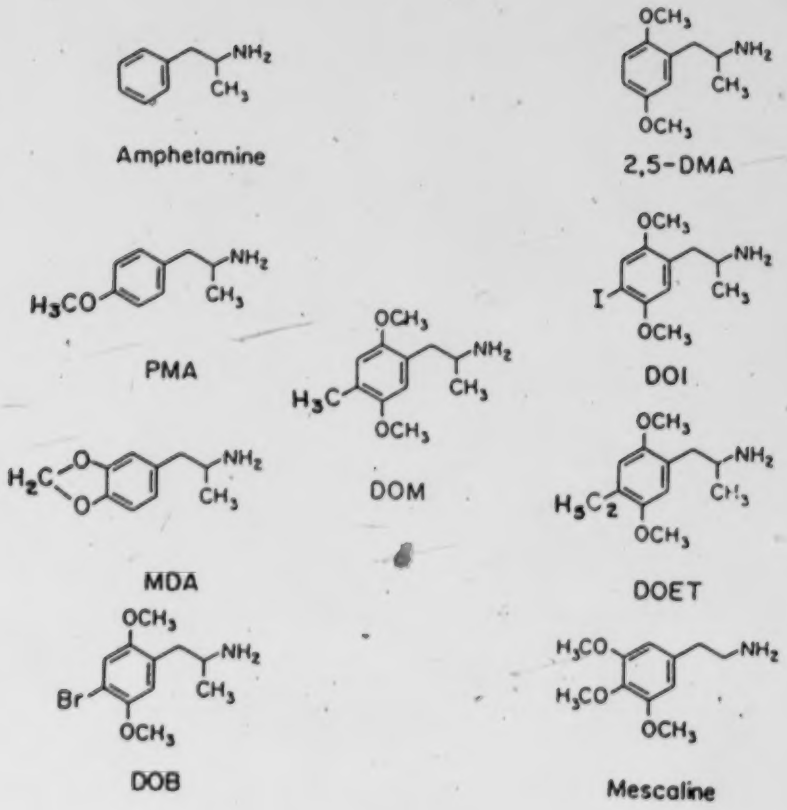


Fig. 1 Structures of some phenethylamines mentioned in the text.

with extracellular recordings from single neurones in vivo. The three types of responses which were studied have been identified using this electrophysiological technique (Aghajanian, 1981) and named S_1, S_2 and S_3 (a classification which is not widely used; see Introduction Sections B.1 and E for a discussion of receptor classifications based on the peripheral actions and the central binding of 5-HT).

S_1. This type of 5-HT receptor mediates the facilitatory action of 5-HT on excitatory input to facial (FMN) and spinal motorneurones (McCall and Aghajanian, 1980a; White and Neuman, 1980). The excitatory effects of threshold and subthreshold stimulation of inputs from motor cortex and red nucleus, recorded at the level of the single FMN, are also facilitated by 5-HT acting on this receptor (McCall and Aghajanian, 1979a). These effects of 5-HT appear to be the only ones which are readily and selectively antagonized by small amounts of the classical "D" type antagonists: methysergide, metergoline, cinanserin and cyproheptadine. Fozard (1984), has suggested that these receptors have the characteristics of the 5-HT_2 binding site.

S_2. These responses are obtained from the cell bodies of dorsal raphé (DR) (serotonergic) neurones and are believed to mediate the inhibitory response of DR neurones to 5-HT. These same cells also respond with inhibition to minute doses of intravenous (i.v.) LSD. The effects of both LSD and 5-HT are not prevented by "D" or 5-HT_2 type antagonists (Haigler and Aghajanian, 1974b; Lakoski and Aghajanian, 1985). Recently, the S_2 putative receptor location has been suggested to correlate with the 5-HT_{1A} binding site. This is

partly based on the binding of, and inhibitory responses to, 8-hydroxy-2-(di-n-proplylamino)-tetralin (8-OH-D-PAT, a 5-HT_{1A} ligand) on rat DR neurones (de Montigny et al., 1984; Vergé et al., 1985; Sprouse and Aghajanian, 1985). DR cell body receptors do not appear to be the same ones that regulate the release of 5-HT at the terminals of DR neurones (see Section E of the Introduction). Finally, the finding that LSD is not an agonist on FMN (S_1) receptors (McCall and Aghajanian, 1980b) argues against any similarity of the S_1 receptor with a form of 5-HT_1 site.

S_3. S_3-mediated responses are obtained from the majority of forebrain neurones which are inhibited by 5-HT, an effect which is independent of the cells' firing rate. Roberts and Straughan (1967) found that 30% of cortical cells are excited by 5-HT. These responses may depend upon the firing rate of the cell, and therefore membrane potential, as is the case with cerebellar cells (Strahlendorf et al., 1984) and may be mediated by separate receptors from those mediating pure inhibition. The most likely receptor candidate to mediate cortical excitatory responses would be the S_1 (or 5-HT_2 site) receptor, as these effects are prevented by "D" type antagonists. The evidence that the pure inhibitory responses of forebrain neurones to 5-HT are mediated by mechanisms (receptors?) different from those of S_1 receptors is clear. This evidence is centered on the finding that S_3 responses are not prevented by the classical "D" type antagonists. The evidence that S_3 receptors are

different from S_2 receptors rests upon the relatively lower potency of LSD on S_3 receptors relative to S_2 receptors (Haigler and Aghajanian, 1974a; de Montigny and Aghajanian, 1977; Wang and Aghajanian, 1977). However 8-OH-D-PAT, which labels the S_2 ($5HT_{1A}$) receptor on DR neurones, will also bind to hippocampal dentate gyrus cells (Vergé et al., 1985) and hyperpolarize CA_1 pyramidal cells (Andrade and Nicoll, 1985).

B. Peripheral 5-HT receptors

The original classification of peripheral 5-HT receptors was based upon the action of 5-HT in the guinea-pig ileum. In this tissue, 5-HT acts upon receptors named "M" and "D" types (Gaddum and Picarelli, 1957). The basis of this classification was that morphine and phenoxybenza-mine (dibenzyline) were able to block the action of 5-HT on nerves and muscle respectively. This terminology has been criticized, as morphine probably prevents contractions to 5-HT by acting as an antagonist of the release of ACh, and phenoxybenzamine is not a specific 5-HT antagonist (see Fozard, 1984). The "M" receptor is neuronal, and is only activated by ring-hydroxylated indoles; it is not blocked by 5-HT antagonists such as methysergide. The "D" receptor is located on smooth muscle, can be activated by non-ring hydroxylated indoles such as tryptamine, and is readily and selectively blocked by methysergide (Drakontides and Gershon, 1968).

Most excitatory responses to 5-HT on central neurones (Davis et al., 1985), motor neurones (McCall and Aghajanian, 1980a; White and

Neuman, 1983) and preganglionic sympathetic neurones (McCall, 1983) are prevented by methysergide (a "D" receptor antagonist). Whilst in most cases the "D" and 5-HT_2 receptors are the same (Bradley et al., 1983); the "D" receptor is not always synonymous with the ketanserin or 5-HT_2 binding site, because in guinea-pig ileum methysergide is an effective antagonist but ketanserin is not (Van Nueten et al., 1983). Furthermore, there are neuronal excitatory responses in the periphery which are not antagonized by "D" receptor antagonists. These "D" receptor antagonist-resistant responses can be found on postganglionic sympathetic and parasympathetic fibres, as well as afferent and enteric neurones (reviewed by Fozard, 1984). All these responses, except those mediated by the classical "M" receptor (postganglionic parasympathetic neurones of the guinea-pig myenteric plexus), are antagonized specifically by MDL-72222, a tropine ester of cocaine. Recently, Richardson et al. (1985) have developed a group of compounds which bear a structural resemblance to both 5-HT and cocaine. These indoletropanyl esters appear to be more potent than MDL-72222 on the same peripheral neuronal "M" receptors. They also block (with less potency) the classical "M" receptor of guinea pig ileum. However, none of the "M"-antagonists so far synthesized has shown appreciable affinity for "D" receptors, 5-HT_1 or 5-HT_2 binding sites. Interestingly, there are few clear reports of the selective antagonism of the inhibitory effects of 5-HT in the periphery (Fozard, 1984). The different classification systems that have been used for 5-HT receptors are shown in Table 1.

TABLE 1. Different serotonin receptor classifications

IN VITRO PHARMACOLOGY (Peripheral)			BINDING (Central)			ELECTROPHYSIOLOGY (Central)		
Receptor	Antagonists	Tissue	Site	Ligand	Location	Receptor	Antagonists	Location
"D"	Phenoxybenzamine "D" antagonists: Cinanserin Cyproheptadine Methysergide	Smooth[a] Muscle	5-HT$_2$	Spiperone[d] Ketanserin	Nerve Blood[e] vessels	S_1	"D" antagonists	Facial motor nucleus[h]
"H"	(Morphine) ICS 205-903	Nerve[a,b] enteric	5-HT$_{1A}$	8-OH-D-PAT[f]	Dorsal[i] raphé	S_2		Dorsal[h] Raphé
"H_P" "H_S" "H_A"	MDL 72222 ICS 205-903	Nerve Parasym-[c] pathetic Sympa-[b] thetic Sensory	5-HT$_{1B}$[g]			S_3		Hippocampus[h] Amygdala V. Lateral-Geniculate-Nucleus.
INHIBITION -			NO ANTAGONISTS!					

References

a Gaddum and Picarelli, (1953); b Richardson et al. (1985); c Fozard, (1984); d Peroutka and Snyder, (1979); e Leysen et al. (1984); f Middlemiss and Fozard, (1983) g Nelson et al. (1981); h Aghajanian, (1981); i Vergé et al. (1985).

The actions of hallucinogens on smooth muscle in vitro

The response of isolated tissues to LSD was first studied by Gaddum in 1954. In this paper (Gaddum and Hameed, 1954) it was reported that LSD (0.6 μg/l) antagonized the contractile effect of 5-HT on the rabbit ear artery and rat uterus preparations. Since then Feniuk (1984) has suggested that 5-HT interacts with only α-adrenoceptors on rabbit ear arteries. Gaddum and Hameed (1954) suggested that the ability of LSD to antagonize 5-HT could be the basis of the drug's mental effects in humans. Almost immediately this hypothesis was pronounced unlikely (Cerletti and Rothlin, 1955) as it was shown that Brom-LSD, which blocked 5-HT on rat uterus, was devoid of potent psychic effects in humans. In 1956 Costa (1956) examined the actions of low doses of LSD on the rat uterus and noted sensitization of agonist effects; similar findings have been noted in most in vitro preparations (Freedman and Halaris, 1978). A partial agonist profile is often overlooked in discussions of the peripheral antagonist actions of LSD on "D" type smooth muscle 5-HT receptors.

In 1974 Cheng et al. (1974a) studied the actions of methoxylated amphetamines on the dog dorsal metatarsal vein. All the compounds tested produced a long-lasting contraction. Those produced by PMA, 2,4-DMA, 2,5-DMA, 3,4-DMA and (+)-DOM were reduced by phentolamine, whereas those produced by (-)-DOM, (±)-, (+)- and (-) DOB were not. Responses to all forms of DOM and DOB (but not the others) were antagonized by cinanserin, a "D" type 5-HT antagonist. The authors

concluded that PMA, 2,4-DMA and 3,4-DMA were indirect agonists which released NA onto α-adrenoceptors, but 2,5-DMA stimulated these receptors directly. Finally, (+)- and (-)-DOM and all forms of DOB directly activated 5-HT receptors. In this study it was also noted that the S(+) isomers of DOM and DOB were more potent than the R(-) isomers in their ability to activate 5-HT receptors.

It is interesting to note that it is the R-(-) isomer of DOM which is the most potent psychotomimetic (Shulgin, 1973). In the sheep umbilical artery, however, it is the R-(-) isomers of DOM and DOB which are the more potent 5-HT receptor agonists (Dyer et al., 1973). In the umbilical artery (which is not innervated) the actions of DOB, DOET, DOM and 2,5-DMA were blocked by Brom-LSD but not by atropine or piperoxan. Cross protection against phenoxybenzamine blockade, was found to occur between 5-HT and (-)-DOM. Dyer (1973) went on to demonstrate that LSD, mescaline and bufotenine contracted sheep umbilical arteries. 5-HT protected its own receptors from blockade by phenoxybenzamine, it also prevented the blockade of responses to mescaline, LSD and bufotenine. Dyer and Grant (1972) also showed that responses of human umbilical arteries to bufotenine, psilocin, psilocybin, mescaline and LSD were antagonized by cinanserin and Brom-LSD. It was also found that these responses were not due to activation of adrenergic, cholinergic or histamine receptors. In 1976 Dyer (1976) further investigated the action of DOM on the umbilical artery and concluded that it was a pure agonist. Evidence for species

variation in the sensitivity of umbilical arteries to hallucinogens and 5-HT antagonists was obtained by Nair (1974). Guinea-pig umbilical arteries were less sensitive to mescaline than were sheep and human arteries. Whilst the responses were antagonized by Brom-LSD, the response to LSD was somewhat resistant. Cinanserin did not prevent responses to LSD, psilocin or psilocybin.

LSD, mescaline and phencyclidine are also potent contracting agents of cerebral arteries (Altura and Altura, 1981). 5-HT antagonists were also evaluated on this preparation: methysergide prevented LSD- and mescaline-, but not phencyclidine-induced contractions. It is noteworthy that hallucinogens behaved as pure agonists on the umbilical artery, cerebral artery, and metatarsal vein.

Another peripheral tissue containing 5-HT receptors is the rat stomach fundus. This tissue has been used as a model on which to examine the effects of phenethylamine hallucinogens. The preparation was originally developed by Vane (1957), and contains 5-HT receptors which when activated elicit a contraction of the fundus. The model is complicated by the existence of two types of 5-HT receptors, one sensitive to blockade by phenoxybenzamine and the other which is resistant (Winter and Gessner, 1968). Assuming partial agonist activity, Glennon et al. (1978) have determined "apparent" pA_2 values for hallucinogens acting as antagonists to the contractile effect of 5-HT in the rat fundus. Nichols et al. (1984) evaluated this procedure and were unable to observe any antagonism of 5-HT, even when using a phenethylamine hallucinogen which was reported to have a pA_2

of 6.78 (Glennon et al., 1982a). This value should indicate a fairly potent antagonist. Nichols et al. (1984) suggest that reported pA_2 values for penethylamines all lie in regions of the dose-response curve in which the drugs produce an agonist effect. Despite this difficulty, Nichols et al. were able to antagonise the action of 5-HT with Brom-LSD. Nichols et al. (1984) suggest that it is inappropriate to determine a pA_2 value for a compound with an agonist profile.

Only the D-(+) isomer of LSD is hallucinogenic (Aghajanian et al., 1978). Thus if it is shown that this isomer binds with a greater affinity to the $5\text{-}HT_1$ or $5\text{-}HT_2$ receptor and their equivalents can be identified physiologically, this may provide some evidence about the role the two sites have in the effects of LSD. The phenethylamines show relative stereoselectivity (unlike LSD which is absolutely stereospecific) but the R-(-) enantiomer of DOM is only 2-4 times as potent as the S-(+) enantiomer in humans (Shulgin, 1973). The similarity between the stereochemistry of d-(+) LSD and R(-) DOM was first noticed by Kang and Green (1970). This led to the suggestion that both tryptamine and phenethylamine hallucinogens may interact with the same receptor. However this was before the heterogeneity of brain 5-HT receptors was discovered (Bennet and Snyder, 1975, see Section E of Introduction).

C. Structure-activity relationships of phenethylamines

This section describes studies designed to examine the effects of methoxylated amphetamines on the release, uptake, and metabolism of neurotransmitters. However before this is discussed, it is

appropriate to examine the results of structure-activity relationship studies on the amphetamine molecule with reference to methoxylation, and to compare the results of these studies with predictions made from their structure.

The structural features which determine whether a given phenethylamine can be either a substrate for catecholamine uptake or a uptake inhibitor are: 1) presence of a meta- or para-phenolic hydroxyl group and 2) the absence of a methoxy group (Iversen, 1971). Whilst other findings in this study have been criticized, these rules at least appear to hold true (Maxwell et al., 1976). DOM does not fulfil either of these criteria and so would be expected not to be actively taken up, or to block uptake of NA or DA (Horn, 1973).

Amphetamine accelerates the efflux of neurotransmitters by either enhancing passive diffusion, a carrier-mediated process, or both (Trendelenburg, 1979). The effect of O-methylation on the facilitation of ^{3}H-NA efflux by phenethylamines in rabbit atria was studied by Paton (1975) and Paton and Pasternak (1974). It was found that O-methylation always significantly reduced the ability of compounds to facilitate efflux. PMA and MDA were still able to release transmitters, but the addition of a second methoxy group (2,5-DMA) caused such a great reduction in potency that it became inactive. In the study of Loh and Tseng (1978), the para-methoxylation of amphetamine did not decrease its ability to block the uptake

and increase the release of 5-HT; ortho-methoxylation, however, did decrease potency. At least two studies of DOM and DOM analogues have concluded that methoxylated phenethylamines are very weak inhibitors of 5-HT uptake into rat brain synaptosomes ($I.C._{50}$ 0.1 mM). A similar figure was reached for DA, and NA uptake inhibition had an $I.C._{50}$ of 0.4 mM (Makriyannis et al., 1982; Whipple et al., 1983). On the other hand, PMA was found to be fairly potent at releasing ^{3}H-5-HT ($I.C._{50} < 10^{-6}$ M) from cortical slices, less potent at releasing ^{3}H-NA and very weak at releasing ^{3}H-DA. This was also the case in vivo (Tseng, 1976, 1978 and 1979). Here it is relevant to note that 4-monosubstituted compounds have amphetamine-like effects in man, and, unlike DOM, are not hallucinogenic (Nichols, 1981). As would be expected from the previous statements, it has also been found that ring-methoxylation decreases a compound's central stimulant properties.

No importance of metabolic processes in the mechanism of action of phenethylamine hallucinogens has yet been demonstrated (Ho, 1971). In accord with this, phenethylamines generally are not good inhibitors of monoamine oxidase, and the more active compounds are not good substrates for this enzyme (Clark et al., 1965; Andén et al., 1974). The reasons for this appear to be that an α-methyl group protects the molecule from degradation by monoamine oxidase (Clark et al., 1965) and increases lipid solubility by a factor of three. This is emphasized by the finding that mescaline which has no α-methyl group has poor lipid solubility (Nichols et al., 1977). On the other hand,

addition of an α-methyl group to a methoxylated ring structure increases its potency as a psychotomimetic, probably for the same reason. α-Methylation certainly does not increase the affinity of these drugs for 5-HT receptors as measured in rat fundus strip (Glennon et al., 1980).

Cheng et al. (1974a) proposed that psychoactive phenthylamines could be divided into two groups: those with a direct receptor effect (e.g. the ortho- and meta-substituted DOM, 2,5-DMA, DOB and DOET), and those which stimulate transmitter release (e.g. the para-substituted PMA, MDA and 3,4-DMA). Further support for this concept was obtained recently, when it was found that high concentrations of MDA deplete 5-HT from the rat brain (Ricaurte et al., 1985). Consequently, this class of drugs can be LSD-like, or amphetamine-like, depending on the number and the position of their aromatic substituents. Significantly, those drugs shown to release neurotransmitters have also been shown to have no direct receptor stimulating activity (Glennon et al., 1980). The same holds true for tissues which are not innervated, in which there is no opportunity for indirectly-mediated actions (Nair, 1973; Dyer, 1974). Both PMA and MDA have pronounced cardiovascular effects (Cheng et al., 1974b; Nichols et al., 1975). Their toxicity in the mouse has been compared (Lopatka et al., 1976), and as their effects were blocked by adrenergic neurone blockers, propranolol, phenoxybenzamine and phentolamine, it was concluded that the actions of this class of drugs were largely due to the release of endogenous catecholamines. In cases where DOM was included for

comparison (Cheng et al., 1973; Hwang, 1973; Lopatka et al., 1976; Friedman et al., 1978), the pressor response or mortality was prevented by only methysergide, Brom-LSD or cinanserin. The finding that DOM produces a methysergide-sensitive pressor effect upon i.v. injection, is interesting in the light of the finding that the pressor effect after i.v. 5-HT is probably caused by an action on 5-HT_2 receptors as it is prevented by ketanserin (Leyson et al., 1984).

Further evidence that MDA has a different mechanism of action to DOM is suggested by the finding that N-alkylation of DOM reduced its biologic activity but did not affect that of MDA (Ho et al., 1970; Braun et al., 1980). When animals are trained to discriminate DOM from saline, the DOM stimulus would generalize to R-(-) MDA but not S-(+) MDA (Glennon et al., 1982b). The latter is the compound which is most potent at releasing preloaded ^{3}H-5-HT from rat synaptosomes (Nichols et al., 1982).

D. Effects of indole and phenethylamine hallucinogens on transmitter turnover in the CNS

Leonard (1972) has shown that doses of LSD and mescaline required to affect the rat brain cholinergic system are much higher than those needed to cause hallucinations in man. Similarly high doses of LSD uncouple the action of histamine (at H_2 receptors) on adenylate cyclase (Green et al., 1977) and a range of hallucinogens (including DOM) block H_1 receptors (Fredrickson and Richelson, 1979). Other data, reviewed by Hamon (1984), indicate that anti-histaminergic

activity is not a common feature of all hallucinogens.

The first study of the effect of hallucinogens on central monoaminergic neurones was carried out by Freedman in 1961. He found that a single injection of LSD increased the level of 5-HT in whole brain, while it decreased the concentration of its main metaboli e 5-hydroxyindoleacetic acid (5-HIAA). Brom-LSD, an inactive congener of LSD, did not have this effect. Rosecrans et al. (1967) proposed that LSD slowed the turnover of 5-HT in the CNS. This was confirmed when it was shown that conversion of ^{3}H-tryptophan into ^{3}H-5-HT in brain is slowed by LSD, in other words the synthesis rate is decreased (Lin et al., 1969). LSD also reduces the rate of 5-HT disappearance following 5-HT synthesis inhibition with para-chlorophenylalanine (PCPA) (Andén et al., 1971). Not surprisingly, LSD also decreased the release of ^{3}H-5-HT synthesized in vivo from ^{3}H-tryptophan (Gallager and Aghajanian, 1975). In 1967 Aghajanian (1967) demonstrated that stimulation of the DR nuclei increased 5-HT turnover in the rat brain. A year later Aghajanian et al. (1968) showed that low doses (12 μg/kg) of LSD selectively inhibited serotonergic cells in the DR nuclei. At this time it was suggested that raphé inhibition could be the mechanism of the LSD-induced decrease in 5-HT turnover. However the possible involvement of a receptor mediating DR inhibition could not be shown, due to the lack of an effective 5-HT antagonist (Haigler and Aghajanian, 1974b). One piece of evidence which supports the proposal that raphé inhibition may cause the decreased 5-HT

turnover is that mescaline does not inhibit DR cells (Haigler and Aghajanian, 1973; Trulson et al., 1981). Similarly, it does not decrease 5-HT turnover in rat brain (Andén et al., 1974). However, there is substantial evidence against direct DR cell body involvement in the effects of LSD on 5-HT turnover. Pieri et al. (1978) infused LSD into the dorsal and medial raphé nuclei of rats; this produced no alteration of 5-HT turnover in the forebrain. In addition, Trulson et al. (1981) found that LSD could still inhibit DR firing, even when behavioural and neurochemical tolerance to LSD administration had occurred. Surprisingly, even DR cell body lesions do not prevent the LSD-induced decrease in 5-HT turnover (Halaris et al., 1982). These data suggest that the LSD receptor mediating inhibition of serotonergic neurones is not the one which mediates the decrease in 5-HT turnover. Hamon (1984) suggestedthat LSD cannot inhibit 5-HT synthesis by acting as an autoreceptor agonist on nerve terminals. The reason for this suggestion is that even large concentrations of LSD applied to hippocampal slices do not inhibit the synthesis of 5-HT in them (Hamon et al., 1974). Instead, he has postulated a long negative feedback loop triggered by postsynpatic 5-HT receptors. This concept is supported by the finding that LSD does not inhibit 5-HT synthesis in rats at birth (Bourgoin et al., 1977), when the loops are presumably not formed. Neither is synthesis inhibited in rats whose proposed feedback loops are destroyed by cerebral transection (Kehr and Speckenbach, 1978).

The action of LSD in decreasing ^{3}H-5-HT release may be mediated by an agonist action at 5-HT terminals. Several groups (Chase et al., 1967; Langer and Moret, 1982) have shown that LSD will decrease the K^{+}- or electrically-induced release of ^{3}H-5-HT from brain slices. The effective concentration range of LSD was 0.001-1 μM but L-LSD did not affect this Ca^{++}-dependent 5-HT release, suggesting that this effect may have something to do with its mechanism of action. Methiothepin and metergoline will block the inhibitory effect of 5-HT on its own release (see Section E); these putative antagonists also prevent the LSD effect on the release of 5-HT (Bourgoin et al., 1978; Langer and Moret, 1982). In connection with the LSD feedback action on 5-HT release, Halaris et al. (1982) have found that whole brain increases in 5-HT levels due to LSD were localized in the vesicular fraction of the nerve terminals. A 5-HT binding protein was postulated, which was thought to transport newly synthesized 5-HT to the terminals; it was at this site that LSD was thought to act. Soon after, Gershon (1983) isolated a likely candidate for 5-HT-binding protein. In support of the concept that the blockade of synthesis does not go on at the terminal level, no effect of LSD on the 5-HTP (5-hydroxytryptophan) decarboxylase or the transport system for 5-HTP into the terminals could be found (Halaris et al., 1982).

Several groups have shown that DOM decreases 5-HT turnover and increases 5-HT levels in whole brain (Wallach et al., 1972; Leonard, 1973; Andén et al., 1974). Wallach et al. (1972) found that 8 mg/kg of DOM produced a modest reduction of 5-HT in the cat brain; Leonard

(1973) used 60 mg/kg in the rat and saw a pronounced effect. Andén et al. (1974) used 5-20 mg/kg and noted a profound reduction in 5-HT turnover in the rat brain. Both Andén et al. and Leonard noted that DOM delayed the depletion of 5-HT which occurs after administration of the compounds H22/54 (α-propyldopacetamide which inhibits tyrosine and tryptophan hydroxylase) and H75/77; this is suggestive of effects resulting in depression of 5-HT turnover. Wallach et al. (1972) found a small increase in whole brain NA after DOM, but Leonard (1973) found a decrease in NA levels. This discrepancy may be due to a species difference. The effects of DOM on 5-HT turnover are consistent with reports that DOM is not amphetamine-like, and does not release NA, DA and 5-HT. This result is in extreme contrast to that noted for PMA and MDA (Tseng, 1978). The reason why DOM appears not to release 5-HT may lie in the finding that methoxylation of the ring structure of amphetamines decreases their ability to release monoamines (see Section C of the Introduction).

The serotonergic system is not the only neuronal population affected by LSD: a decrease in cerebral NA levels was reported by Freedman (1963), and tolerance occurred to this effect. Whether this is a consequence of an action of LSD on 5-HT systems is unknown. The possibility that decreased brain NA levels are brought about by an increased turnover of NA is remote. This is because the depletion of rat brain NA induced by the administration of α-methyl-para-tyrosine,

is unaltered by LSD (Leonard, 1972). LSD did however increase DA turnover. This was measured by its effects on DA metabolites and after inhibition of aromatic amino acid decarboxylase (Bowers and Salmonsson, 1982; Persson, 1977).

In conclusion, the findings of Pieri et al. (1978) challenge the assumption that the psychotomimetic actions of LSD correlate with its effects on 5-HT, NA or DA turnover (Brawley and Duffield, 1971). This is based upon the observation that lisuride, a non-hallucinogenic LSD congener, has all of the effects of LSD on turnover and is more potent than LSD.

E. Central binding studies with 5-HT and phenethylamines

In 1973 Farrow and Van Vunakis (1973) showed that ^{3}H-LSD bound with high affinity (K_D = 9 nM) to rat cortical synaptosomes. Several other hallucinogens, but not the non-hallucinogenic L-LSD, inhibited the binding. 5-HT also bound to this site. Bennett and Snyder (1975) confirmed the earlier findings and showed that degeneration of serotonergic neurones did not alter the LSD binding. The conclusion that was drawn from this result was that the binding was postsynaptic. Peroutka and Snyder (1979) went on to find two types of 5-HT binding sites, called 5-HT_1 and 5-HT_2, which were equally labeled by ^{3}H-LSD. The 5-HT_1 receptors selectively bind ^{3}H-5-HT with a high affinity (K_D=1-4 nM). They have a higher affinity for agonists than they do for antagonists. The postsynaptic 5-HT_2 sites are labeled by the antagonists ^{3}H-spiperone (spiroperidol) and ^{3}H-ketanserin, but they have a low affinity for 5-HT (low μM). In

fact most of the "D" smooth muscle receptor antagonists have an affinity for 5-HT_2 sites in the nM range (Peroutka and Snyder, 1979; Leysen et al., 1984). Spiperone was considered to be binding to 5-HT_2 sites and not D_2 (DA) sites as 5-HT antagonists were the most potent displacers of the spiperone. 5-HT, however, was a very weak displacer of spiperone. Nonetheless the ability of drugs to displace ^{3}H-LSD binding correlated very well with their ability to displace spiperone. At the 5-HT_2 binding site LSD displaces 5-HT, LSD, and spiperone with the same K_D (10 nM) suggesting that each ligand binds to the same site. At the 5-HT_1 site, however, 5-HT will displace itself more potently than it will LSD and spiperone.

The 5-HT_2-site correlates with central anti-tryptaminergic activity of 5-HT antagonists (Leysen et al., 1978; Davis et al., 1985), and the pharmacology of the 5-HT "D" receptors in the peripheral vasculature (Bradley et al., 1983; Leysen et al., 1984). Unfortunately, the search for a functional correlate for the 5-HT_1 binding site has run into difficulties. Martin and Sanders-Bush (1982a) have shown an impressive correlation for a few "D" receptor antagonists. Those drugs which bind to 5-HT_1 sites with the highest affinity (metergoline, methiothepin and methysergide) will prevent the inhibitory action of 5-HT on its own K^+-evoked release from prelabeled synaptosomal preparations of rat hypothalamus. The authors concluded that presynaptic 5-HT receptors which mediate feedback inhibition of

5-HT release (Gothert, 1982) may be of the 5-HT_1 type. There are several problems with this conclusion. First, Bennett and Snyder (1975) found that the 5-HT_1 sites were postsynaptic. Secondly, if methiothepin does block a 5-HT presynaptic terminal receptor, thus preventing 5-HT release, this receptor is not the same as the one found on serotonergic cell bodies. At the terminal receptor, 5-HT is 80 times more potent than 5-MeoDMT in decreasing its own release, but 5-MeoDMT is more potent than 5-HT in depressing the firing rates of 5-HT containing DR neurones (de Montigny and Aghajanian, 1977). Thirdly, none of the three antagonists which block the "terminal" effects of 5-HT will block the inhibitory effects of 5-HT. This has been shown on DR cell bodies (Haigler and Aghajanian, 1974b), and at postsynaptic sites in the hippocampus (this thesis) which resemble the terminal site with regard to the relative potencies of 5-HT and 5-MeoDMT (de Montigny and Aghajanian, 1977). Finally, two groups have reported that quipazine blocks the terminal autoreceptor (Martin and Sanders-Bush, 1982b; Schlicker and Gothert, 1981). However, Blier and de Montigny (1983) found that quipazine behaved as a pure agonist on DR cells.

The conclusions of binding studies were complicated when it was discovered that some 5-HT_1 binding sites previously considered to have a low affinity for spiperone actually had a high affinity for spiperone (K_i = 2-13 nM); these were named 5-HT_{1A} binding sites (Nelson et al., 1981). These receptors retained their high affinity

for 5-HT as did the 5-HT_{1B} receptors (which had a low affinity for spiperone K_i = 35 μM). This group showed that the receptor subtypes were found in different areas of the brain; 5-HT_{1A} receptors were found in hippocampus and DR (Deshmukh et al., 1983). Sills et al. (1984) showed that guanosine triphosphate (GTP) will eliminate the high affinity state of this receptor, and suggested that both sites were low and high affinity states of the same receptor.

8-OH-D-PAT is a ligand which has been shown to bind selectively to the 5-HT_{1A} binding site (Middlemiss and Fozard, 1983). Gozlan et al. (1983) have determined that its binding in the rat brain is partially presynaptic, as it is reduced by 5,7-dihydroxytryptamine (5,7-DHT). Marcinkiewicz et al. (1984), using autoradiographic methods, have found that one area of ^{3}H-8-OH-D-PAT binding is concentrated on DR cells. Vergé et al. (1985) have shown that 8-OH-D-PAT binding in the DR is reduced dramatically by 5,7-DHT lesions; however, binding in the terminal regions was not affected. This result suggests that, in terminal regions, 8-OH-D-PAT binds only postsynaptically and not on the raphé terminals. De Montigny et al. (1984) have reported that 8-OH-D-PAT has potent inhibitory effects on DR neurones equivalent to those of LSD. Sprouse and Aghajanian (1985) came to a similar conclusion when they showed that 5-HT_{1A} ligands inhibit DR cell firing, *in vivo* and *in vitro*, but that 5-HT_{1B} ligands are less effective. Vergé et al. (1985) conclude that

receptors on DR cell bodies may be of the 5-HT_{1A} type. However those autoreceptors located on raphé terminals (Gothert, 1982) are neither of the 5-HT_{1A} nor 5-HT_{1B} type.

Clearly, when considering the binding of hallucinogens it becomes important to be able to differentiate the major forms of receptors for 5-HT. If the object is to determine if a drug binds to 5-HT_1 or 5-HT_2 sites, the tissue can be preincubated with 300 nM 5-HT; this should block binding at only the 5-HT_1 sites. Similarly the 5-HT_2 sites can be masked by preincubating the tissue with 30 nM spiperone, thus leaving the 5-HT_1 sites. This approach is inadequate for two reasons. The first is the similarity between D_2 receptors and 5-HT_2 receptors (spiperone will label D_2 and 5-HT_2 sites in the low nM range, Leysen et al., 1978), and the second is the 5-HT_{1A} binding site which will also bind spiperone.

One approach to circumvent these problems may be to include GTP in the medium in attempts to label 5-HT_1 receptors, or to include a small concentration of sulpiride which will bind only to D_2 receptors; the latter procedure is useful in isolating 5-HT_2 binding sites. The 5-HT_2 receptors can be labeled with ketanserin, used in the low nM range; by subtraction procedures one can determine which is 5-HT_2 and which is D_2 binding (Leysen et al., 1979; Altar et al., 1985). Both Kadan et al. (1984) and Nakada et al. (1984) have found that ^{125}I-LSD binds with a high affinity to 5-HT_2 receptors, however D_2 receptor binding accounted for 20-25% of the total binding. Interestingly, if binding from midbrain to frontal cortex is

followed, the ratio of 5-HT_2 to D_2 receptors tends to shift in favour of 5-HT_2 binding (Altar et al., 1985). Others maintain that there are still 20-25% D_2 receptors present in frontal cortex (Marchais et al., 1980).

Using the technique of autoradiography, Meibach et al. (1980) noted that ^{3}H-LSD binding occurred preferentially in hippocampus, particularly in area CA_1 and dentate gyrus. Palacios et al. (1983) localized 5-HT_2 binding sites to layer IV of the frontal cortex, with 5-HT_1 sites in deeper layers. Both of the above groups found a concentration of ^{3}H-LSD binding in the DR nucleus. Kohler (1984) also concluded that the binding sites found in the hippocampus are of the 5-HT_1 type. Binding was postsynaptic, and a high density of 5-HT_1 sites was found in area CA_1. In certain areas, however, correlations between the targets actually innervated by 5-HT terminals and the presence of binding was poor. Seeman et al. (1980) have reported that ^{3}H-LSD binding in the hippocampus increases after specific raphé lesions, but a decrease in binding sites is observed after chronic LSD treatment. Blackshear et al. (1981) did not find any increase in the 5-HT_1 or 5-HT_2 binding sites after raphé lesion, but noted that 25% of pons and midbrain 5-HT receptors were of the 5-HT_2 type. The same group reported (1983) that chronic treatment with drugs that block 5-HT_2 receptors actually decreased the number of 5-HT_2 sites. This is opposite of what one would expect of an antagonist.

De Jong et al. (1982) assessed the ability of several hallucino-

genic amphetamines to prevent the binding of ^{3}H-5-HT to rat brain membranes. In this study, no regional selectivity of binding was examined and no attempts were made to differentiate receptor subtypes. Not surprisingly, DOM (and all other compounds tested) did not have a very high affinity (13 μM I.C.$_{50}$). This problem was tackled with far greater resolution when Shannon et al. (1984) examined the 5-HT$_1$- and 5-HT$_2$-binding properties of a series of methoxylated amphetamines, the majority of which were hallucinogenic. The R-(-)enantiomers of these drugs, which are slightly more potent hallucinogens than the S-(+) (Shulgin, 1973), were the more potent displacers of ketanserin, and showed selectivity for 5-HT$_2$ sites. The interactions with 5-HT$_1$ sites were with a lower affinity and a lack of stereoselectivity. Later work by Glennon et al. (1985) found high correlations between 5-HT$_2$ binding affinities and human hallucinogenic potencies for 15 hallucinogens. Whitaker and Seeman (1977) have found that some indole and phenethylamine hallucinogens (DOM) will prevent the binding of 3.3 nM haloperidol to the rat caudate. Concentrations required were less than 100 nM, but it was pointed out that the same drugs (with the exception of methysergide) would not prevent the binding of 1.5 nM apomorphine. There are several interpretations for these results: one is that the hallucinogens bind to DA/neuroleptic receptors; another is that a portion of haloperidol binding in the caudate is to receptors for serotonin. The latter is the possibility favoured by Leysen et al. (1978) and Altar et al. (1985).

When ^{3}H-DOM is injected intravenously in adult cats it is interesting that DOM (measured by autoradiography) concentrates in the hippocampus, amygdala, hypothalamus and geniculate bodies (Idanpaan-Heikkila et al., 1969). In a study by Ho et al. (1971), no appreciable metabolites of DOM were found; the unchanged compound was measured in the brain with a similar distribution to that seen for LSD autoradiography. An interesting difference between the rat and the monkey was noted, in that 8% of the applied dose was the maximum found in monkey brain, but only 1% was found in rat brain. This may account for the high doses of DOM required (by the intravenous route) to perturb 5-HT turnover in the rat, relative to the cat (see Section D of Introduction).

F. Behavioural Pharmacology of DOM

1. Effects of hallucinogens on conditioned behaviour

The effects of hallucinogens on conditioned and unconditioned behaviour in animals have recently been reviewed (Davis et al., 1984; Appel and Rosecrans, 1984). For this reason protocols which throw most light on the neuropharmacology of these drugs will be briefly discussed, along with some results obtained with DOM.

Conditioned avoidance responding of the rat is disrupted by hallucinogens (Davis et al., 1978). It has also been found that PMA and MDA (see Fig. 1) have a different neurochemical and pharmacological profile to DOM or indoleamine hallucinogens (Tseng, 1979). In view of this, the conditioned avoidance paradigm should

demonstrate differences between the effects of PMA and MDA, and DOM (a direct agonist). Little work has been done in this area. The only report available shows that D-amphetamine and DOM can be distinguished (Davis et al., 1978). There is one report, however, that the disruptive effects of DOM on conditioned avoidance can be antagonized by the 5-HT antagonist cinanserin (Tilson et al., 1975).

In schedules of reinforced responding, low doses of DOM and most other hallucinogens cause a pause in the response maintained by food (Freedman et al., 1964). The effects of hallucinogens which cause pausing are selectively antagonized by "D" type 5-HT antagonists (Winter, 1978). This technique can be adapted so that in a drug discrimination study the animal can in effect tell us which drug it "thinks" it has been given. Glennon et al. (1979) have shown that DOM and DOET responding showed partial generalization to 5-MeoDMT (an indole hallucinogen) rather than saline. Silverman and Ho (1980) could transfer DOM responding to mescaline but not to D-amphetamine; this suggests that the DOM and mescaline cues are similar. In all cases hallucinogen stimulus transfers are prevented by only 5-HT antagonists (Kuhn et al., 1978), although 5-HT antagonists can occasionally produce hallucinogen-appropriate responding (Colpaert et al., 1982). It is also noteworthy that mescaline and DOM cues are readily antagonized by cinanserin (Browne and Ho, 1975). However LSD and 5-MeoDMT cues are only prevented with higher doses (Kuhn et al., 1979). Similarly, metergoline prevented LSD and N,N-dimethyl-

tryptamine effects on fixed ratio responding, but shifted the dose-response curve for DOM further to the right than it shifted responses to the other drugs (Commissaris et al., 1981).

2. Hallucinogens and unconditioned behaviours

In rodents at least, locomotor activity is generally decreased by increasing levels of brain 5-HT. Conversely, hyperactivity is produced by treatments that reduce brain 5-HT, such as PCPA, para-chloroamphetamine, raphé lesions or 5,7-DHT (Gershon and Baldessarini, 1980). Brody's work (1970) suggests caution in the application of this generaliza-tion. He proposes that the 5-HT system does not control the level of locomotor activity, but that it may control the sensory responsivity of the animal to environmental stimuli. If this is so it may explain discrepancies in results from different experimental protocols.

Jacobs and Trulson (1976) reported that LSD and other hallucinogens caused unusual behaviour in cats: namely limb flicking, abortive grooming and investigatory play behaviour. These responses were claimed to be specific for hallucinogens given in the human dose range (2.5-200 μg/kg LSD) and were found to be centrally mediated. These behavioural effects roughly followed the time course expected of LSD-induced behavioural effects and showed the development of tolerance. Other drugs which induce limb flicking are psilocybin, psilocin, N,N-dimethyltryptamine, DOM and mescaline (Rusterholz et al., 1977). In these initial studies, several non hallucinogenic compounds (Brom-LSD, D-amphetamine, tryptamine, atropine, caffeine and

chlorpheniramine did not induce limb flicks. However, later investigations found that non-hallucinogens would cause limb flicking: methysergide, lisuride (Marini et al., 1981a), apomorphine and quipazine (White et al., 1981). It was also found that prior depletion of 5-HT will potentiate the behavioural effects of LSD (Trulson et al., 1976). This could be due to the development of supersensitive 5-HT receptors or a possible reduction in the amount of 5-HT competing for the 5-HT/LSD receptor.

Jacobs and Trulson (1978) recorded from DR serotonergic cells of the cat, and noted that DR activity was proportional to the frequency of the LSD-induced limb flicks. In their paper, Trulson et al. (1979) found that low doses of LSD often produced only small decreases of DR activity but significant behavioural changes. Furthermore, LSD-induced behaviour outlasted the depression of the DR, and DR neurones were just as responsive to LSD during tolerance as in the non-tolerant (behaviour) condition. More evidence emerged in 1979 which decreased the value of these correlations. Lisuride is an LSD congener which decreases brain 5-HT turnover (Pieri et al., 1978). Lisuride inhibited DR cell firing when given by the intravenous route or by microiontophoresis; it was 5-10 times as potent as LSD (Kogawski and Aghajanian, 1979). Unlike limb flicks induced by LSD, tolerance did not develop to the lisuride-induced flicks (Marini et al., 1981a). In order for the model to be specific for hallucinogens it was proposed that all hallucinogen-induced limb flicking would

develop tolerance. However the modified theory was not long unchallenged.

Marini and Sheard (1981b) reported that methysergide caused significant limb flick behaviour which developed tolerance and cross tolerance with LSD effects. Despite the good correlations found between 5-MeoDMT effects on DR unit activity and the time course of limb flicks, the same correlations did not hold for LSD. When we remember that the phenethylamine hallucinogens are poor inhibitors of the DR (this study), it becomes clear that raphé inhibition is not sufficient nor necessary for the production of limb flicks in cats. Limb flicks may be mediated by an action on excitatory 5-HT receptors, possibly 5-HT_2, as they are antagonized by methysergide, mianserin and cyproheptadine (Marini and Sheard, 1981b).

Early views of the role of 5-HT in behaviour were that increased levels of 5-HT given intracerebroventricularly (i.c.v.) were associated with sedation, but that large amounts caused excitation (Mantegazzini, 1966). Serotonergic neurones were thought to oppose the arousing effect of pontomesencephalic catecholaminergic neurones, t'us initiating slow-wave sleep (Jouvet, 1969). This issue is controversial because other neurotransmitters are almost certainly involved (Krnjevic, 1974).

More recent literature has described a variety of behavioural effects that are presumably caused by increased serotonergic transmission in the central nervous system. These effects include tremor, rigidity and hypertonus, reciprocal forepaw treading, hindlimb

abduction, straub tail, lateral head weaving, head and body shakes, salivation, hyperactivity and hyperreactivity. The ability of hallucinogens to cause this "5-HT syndrome" has been well studied, and all hallucinogens produce it (Sloviter et al., 1980; Yamamoto and Ueki, 1981; Niemegeers et al., 1983).

Deakin and Dashwood (1981) have reported that the syndrome can be subdivided, in that hyperactivity and hyperreactivity have a catecholamine link. These behaviours were the only ones prevented by prior pretreatment with α-methyl-para-tyrosine. There is extensive evidence that the other components are mediated by 5-HT receptor activation. Approaches which have been used include work on 5-HT releasing agents, 5-HT precursors, 5-HT uptake blockers, and receptor agonists. The syndrome induced by 5-HTP can be prevented by pretreatment with the 5-HT synthesis inhibitor PCPA, whereas α-methyl-para-tyrosine was without effect except on hyperactivity and hyperreactivity (Sloviter et al., 1980). Tryptophan loading, in conjunction with a MAOI produces the syndrome (Green and Grahame-Smith, 1976). This effect is potentiated by 5-HT uptake blockers (Hwang and Van Woert, 1980) or the destruction of serotonergic nerve terminals by pre-treatment with 5,7-DHT (Jacobs and Eubanks, 1976). Central L-aromatic amino acid decarboxylase inhibitors also block the development of the syndrome, by preventing the conversion of 5-HTP to 5-HT (Sloviter et al., 1980). 5-HT would only produce the syndrome when given i.c.v. (centrally). If an animal pretreated with a MAOI is given L-3,4-dihydroxyphenylalanine (L-dopa)

(or DA i.c.v.) the syndrome will result; this is probably due to indirect 5-HT receptor activation (Sloviter et al., 1980; Deakin and Dashwood, 1981). The reasoning behind this statement rests on the finding that the syndrome is blocked by PCPA or 5,7-DHT but not α-methyl-para-tyrosine, and by 5-HT antagonists but not DA antagonists. Doses of drugs that produce the 5-HT syndrome are usually 100 times greater than those required to inhibit DR firing. However it is interesting to note that mescaline does not inhibit, and DOM poorly inhibits the DR, but both cause the syndrome (Sloviter et al., 1980). The finding that parts of the syndrome (e.g. head twitch and forepaw treading) are antagonized by 5-HT antagonists suggests that 5-HT receptors which mediate excitation may be involved. Although α-adrenergic, dopaminergic, and cholinergic antagonists do not prevent the syndrome, other agents not normally known for their 5-HT blocking potency (e.g. propranolol, morphine, amitryptyline, chlorpromazine and clonidine) do. Niemegeers et al. (1983) have suggested that the antagonism of the syndrome by miscellaneous drugs may be due to an indirect interaction with 5-HT neurones or their output. This conclusion is based upon the good correlations seen between the ability of drugs to block mescaline-induced head twitches (5-HT_2) and also block the syndrome. Such correlations were not seen with drug effects on other behaviours.

The action of hallucinogens on spinal reflexes has been reviewed by Davis et al. (1984) who concluded that hallucinogens facilitate

spinal mono- and poly-synaptic reflexes in both flexor and extensor muscles. Whilst facilitation of this reflex can be caused by several drugs or transmitters, facilitation induced by hallucinogens is usually prevented by 5-HT antagonists and so is probably mediated by an excitatory "D" or 5-HT_2 type receptor (see review by Davis et al., 1984).

Andén et al. (1974) have studied the actions of phenethylamine hallucinogens on the hindlimb extensor reflex in the acutely spinalized rat. DOM produced an increase in the reflex which lasted several hours. No 5-HT antagonists were used, but 5-HTP and tryptophan alone or with a MAOI produced the same effect. The 5-HTP-induced facilitations were blocked by a central decarboxylase inhibitor, but not by phenoxybenzamine or haloperidol pretreatment. Surprisingly, mescaline unlike DOM, did not facilitate the reflex. The final noteworthy point from this study was that effects of DOM were independent of presynaptic stores, just as are its behavioural effects (Wallach et al., 1972).

As some groups of raphé neurones are tonically active (Aghajanian and Wang, 1978), the effects of hallucinogens in the intact animal would be expected to be more complex than in the spinal animal (indole hallucinogens inhibit DR firing). These drugs probably remove tonic facilitation or inhibition in addition to their effectsupon post-synaptic receptors. The work of Trulson et al. (1983b) has a bearing on this proposal; they found that hallucinogens have little

effect on the nucleus raphé pallidus (B1) which probably projects to the ventral horn (Bowker et al., 1981). As an indication of the net effect on monosynaptic reflexes in man it has long been known that indole hallucinogens facilitate the knee jerk reflex (Rosenberg, 1963).

G. Microiontophoretic studies with 5-HT and hallucinogens

One important approach when attempting to assess whether a compound has agonist effects on a certain receptor involves the use of antagonists. Attempts to find a specific antagonist of the actions of 5-HT on central neurones started 23 years ago. The early studies have been reviewed by Bloom et al. (1972) and Krnjevic (1974). It was generally found that in a variety of brain areas LSD did not specifically antagonize the inhibitory actions of 5-HT.

1. Effects of antagonists on 5-HT-mediated inhibition

One of the first reports of the successful antagonism of a central inhibitory response to 5-HT was that of Tebecis (1970). In this study strychnine prevented the inhibitory action of 5-HT on some thalamic cells. Two years later Tebecis (1972) reported that methiothepin prevented the inhibitory action of 5-HT on lateral geniculate neurones (LGN), while responses to γ-aminobutyric acid (GABA) and DA were unaffected. Haigler and Aghajanian (1974b) studied cells in the DR, ventral LGN and amygdala. Cinanserin, cyproheptadine, metergoline, methysergide and methiothepin were tested and a complete lack of antagonist effects on 5-HT inhibition was found. The putative antagonists actually potentiated the effects of 5-HT. There were two major differences between the experiments

carried out by these two groups: first, Tebecis worked on cats and the others worked on rats; secondly the cat cells studied were in the dorsal LGN and the rat cells were in the ventral LGN.

Biscoe and Straughan (1966) reported that spontaneously active, ACh-maintained, and GLU-maintained hippocampal CA_1 pyramidal cell firing was inhibited by 5-HT. This finding was reproduced in 1975 and 1976 by Segal. Segal (1976) also found that LSD, Brom-LSD, cyproheptadine and methysergide had an antagonist action on these responses to 5-HT before a local anaesthetic effect occurred. The inhibitory effect of median raphé stimulation on CA_1 cells was also prevented by methysergide: however cinanserin, methiothepin and mianserin produced only inhibitory responses. Finally, picrotoxin partially prevented the inhibitory response to 5-HT, a finding which was also reported by Mayer and Straughan (1981). When CA_1 cells were recorded from intracellularly in vitro (Segal, 1980), it was found that 5-HT produced a small hyperpolarization associated with a decrease in input resistance. The effects of 5-HT on input resistance were blocked by methysergide, but the hyperpolarization was not completely blocked. Concentrations of methysergide required to produce this effect were very large (0.1 mM) relative to the 5-HT concentration. In addition, specificity of the methysergide effect, relative to its effect on other inhibitory putative neurotransmitters was not assessed.

Wang and Aghajanian (1977a) reported that only following

intravenous administration was LSD able to prevent depression of amygdala neuronal firing by DR stimulation. Aghajanian has suggested that since LSD has an inhibitory effect upon DR cells, it probably also reduces their excitability and this would result in an increased threshold for electrical excitation (Aghajanian and Wang, 1978). Some support for this explanation came with the finding that the LSD-induced blockade of amygdala inhibition, in response to DR stimulation, could be overridden by increasing the current of the DR stimulation. This indirect blocking action of antagonists should be borne in mind in all studies where 5-HT antagonists reduce raphé stimulation-induced postsynaptic inhibition (Briggs, 1977; Dray and Oakley, 1977; Sastry and Phillis, 1977a; Davis and Tongroach, 1978; Jones, 1981; Olpe, 1981). Dray and Oakley (1977) reported that methiothepin reduced median raphé-evoked and exogenous 5-HT-induced inhibition of single substantia nigra neurones. Methiothepin, however, was not completely selective for 5-HT when tested against DA-mediated inhibitory responses. Similar results were also obtained with methysergide (Davis and Tongroach, 1978). Sastry and Phillis (1977b) reported that metergoline selectively reduced the inhibitory effects of 5-HT on cortical cells, but the selectivity of the antagonism relative to NA or DA was not established. However, Jones (1981, 1982) reported that metergoline was not a very effective antagonist of cortical inhibitory responses to 5-HT although it

prevented responses to tryptamine at lower currents. In the same study methysergide did not prevent inhibitory, but only excitatory, responses to 5-HT. Bradshaw et al. (1983) came to the same conclusion regarding the the action of methysergide on inhibitory responses to 5-HT in the rat cortex.

There have been at least three studies of the action of putative 5-HT antagonists on hippocampal field potentials in vitro (Beck and Goldfarb, 1985a; Beck et al., 1985; Olpe et al., 1985; Rowan and Anwyl, 1985). These studies do not form a consistent body of evidence. In all cases a large concentration of 5-HT was shown to reduce the CA_1 population spike amplitude elicited by stimulation of the stratum radiatum. Similarly, it was shown that 0.1 mM methysergide or 0.01 mM cyproheptadine (with a lower 5-HT agonist concentration) prevented the effect of 5-HT. Beck et al. (1985) found that spiperone was an effective antagonist but that ketanserin was not. If both drugs were acting at 5-HT_2 receptors both would be expected to work, although spiperone also has a high affinity for 5-HT_{1A} receptors (see Section E of Introduction). In contrast, Rowan and Anwyl (1985) found that ketanserin (7.5 μM) was an effective antagonist but that antagonism took 30-60 min to occur. As the relationship between field potentials in vitro and spontaneous or induced firing in vivo is not clear, antagonism may represent the prevention of an excitatory effect of 5-HT at a site other than the pyramidal cell soma. The response is complicated because in addition

to the effect of 5-HT on the population spike, the positive wave of the field potential was also attenuated. Finally, in all these studies the specificity of the antagonism was not assessed against other inhibitory neurotransmitters.

Lakoski and Aghajanian (1985) have recently published work which shows that ketanserin, the 5-HT_2 ligand and "D" antagonist, does not prevent the inhibitory response to 5-HT of single cells in prefrontal cortex, lateral geniculate and DR nucleus, but often specifically potentiates 5-HT-induced inhibitions. This and other work (Haigler and Aghajanian, 1974b) is in sharp contrast to the report of Yoshimura and Higashi (1985), who found that low concentrations of LSD and methysergide prevented 5-HT- and stimulation-induced hyperpolarizations of DR neurones in vitro. The conclusions of this work would be strengthened by some demonstration of the degree of specificity of the antagonism of 5-HT.

Generally, it is tempting to conclude that the literature provides little supporting evidence for the suggestion that 5-HT-mediated depressant actions on central neurones can be consistently and selectively antagonized.

2. Effects of antagonists on 5-HT-mediated excitation

Roberts and Straughan (1967) reported that in the cat 30% of cortical cells were excited by the iontophoretic application of 5-HT; LSD, methysergide and cinanserin antagonized the excitatory effect of 5-HT on half the cells tested. Antagonism of 5-HT

could still be seen when GLU- and ACh-induced excitations were unaffected. Using decerebrate animals, Boakes et al. (1970) reported that 5-HT excitated many randomly enountered bulbar reticular formation cells. Rather large currents of LSD were found to block this excitatory effect of 5-HT; similar results were obtained with intravenous drug. However, these amounts of LSD also interfered with the excitatory effect of GLU, but not that of ACh. Iontophoretically applied methysergide was found to be less effective as a 5-HT antagonist than was LSD, and Brom-LSD was usually inactive. Bradley and Biggs (1974) reported that the indole hallucinogens DMT, 5-hydroxydimethyltryptamine (5-OH-DMT) and 5-MeoDMT all reduced 5-HT-induced excitations, unlike the non-hallucinogen 5-methoxytryptamine (5-MeoT). These indoles differed from LSD as they rarely blocked GLU and they were able to mimic 5-HT, with 5-MeoT being the most effective. The excitatory effects of the indoles were not abolished by PCPA or reserpine, suggesting a direct effect on 5-HT receptors. Doubt has been cast upon the physiological relevance of the responses of unidentified cells of the bulbar reticular formation for two reasons: the first is that very high currents of all drugs are required to produce responses (Aghajanian et al., 1978). Secondly, Fuxe (1965) had concluded that there were few 5-HT terminals in the bulbar reticular formation. In 1974 Palkovits suggested that there were in fact fine 5-HT-containing processes coursing into the bulbar reticular formation. In 1977 Briggs stimulated the bulbar raphé nuclei and observed excitation of bulbar reticular formation

cells, which was prevented by i.v. or iontophoretically applied LSD. Other workers have observed similar phenomena; Couch (1970) reported that LSD antagonized the excitatory effect of 5-HT on pontine raphé cells. The one conclusion that seems to be uncontested is that LSD has never been reported to mimic the excitatory effects of 5-HT.

McCall and Aghajanian (1979a, 1979b, 1980a, 1980b) have described the response in vivo of FMN's to 5-HT and NA. They found that large currents of 5-HT and NA did not cause FMN's to fire action potentials. If on the other hand there was an excitatory input (either from stimulation of a synaptic input, or the leakage of GLU from the iontophoretic electrode) then small currents of both transmitters produced large excitatory responses. This group went on to show that the response to 5-HT became supersensitive after denervation, and that a range of smooth muscle "D" type antagonists selectively antagonized the 5-HT-mediated excitation.

Some hallucinogens were found to potentiate responses to 5-HT and NA, but the effect of DOM was not tested. Interestingly, 5-MeoDMT and mescaline caused 5-HT agonist-like effects. Vandermaelen and Aghajanian (1980, 1982a, 1982b) recorded intracellularly from FMN units in vivo and showed that 5-HT and 5-MeoDMT caused a small depolarization which was slow in onset and was accompanied by an increase in input resistance. When the cell was given a depolarizing pulse (2/3 of rheobase) in the presence of 5-HT and NA, spiking was

induced; the response to 5-HT was selectively antagonized by methysergide. Similar excitatory responses to 5-HT and NA have been recorded in the rat spinal cord. These responses were also antagonized selectively by methysergide and metergoline (Barasi and Roberts, 1974; White and Neuman, 1980, 1983; Todd and Millar, 1984). It has been suggested that central neuronal excitatory responses to 5-HT are prevented by ketanserin, the 5-HT_2 ligand (Davis and Roberts, 1985). To date there seems to be good agreement that excitatory effects of 5-HT are readily and reversibly antagonized by low currents (and i.v. doses) of "D" type peripherally effective 5-HT antagonists. These antagonists are the ones that bind with the highest affinity to the 5-HT_2 or ketanserin binding site (Leysen et al., 1984).

3. The effects of hallucinogens on serotonergic cells

Dahlstrom and Fuxe (1965) demonstrated that the origins of many 5-HT fibres in the brain were in the various subgroups of the raphé nuclei. At this time it was known that low i.v. doses of LSD decreased 5-HT turnover (Freedman, 1961). Inspired by this finding Aghajanian et al. (1967) showed that stimulation of the midbrain raphé nuclei of the rat increased 5-HT turnover in the brain. Following this, in 1968 he demonstrated that the DR was inhibited by very low doses of i.v. LSD (Aghajanian, 1968). Brom-LSD was fairly ineffective at inhibiting DR units, but DMT and psilocin were quite potent. Haigler and Aghajanian (1974a) suggested that the hallucinogens which inhibited the DR were less potent on the terminal field cells in terms

of average iontophoretic current needed for 50% inhibition of firing rate. Although firing rates in the different terminal regions were different, 5-HT was approximately equipotent at inhibiting the raphé- and the terminal-located cells. Foote et al. (1969) reported that i.v. application of the phenethylamines mescaline and DOM inhibited a ventral subgroup of DR neurones, but that D-amphetamine caused excitatory responses. Following up on these observations, Haigler and Aghajanian (1973) compared the effects of i.v. mescaline with those to iontophoretically applied drug. As before, mescaline inhibited a ventral subgroup of the cells when applied by the i.v. route, however, no correlation with this response could be found when the drug was applied directly to the cells. Unlike LSD, mescaline was solely a local anaesthetic. On the basis of this result, the effects of intravenous mescaline on DR cells were judged to be indirectly mediated. Due to the finding of a prererential affinity of hallucinogens for presynaptic DR receptors (Haigler and Aghajanian, 1974a; de Montigny and Aghajanian, 1977), it was suggested that drugs in this class may "shut down" the brain 5-HT system. The general effect of 5-HT in the brain was believed to provide an inhibitory tonic control of the higher centres (Bloom et al., 1972), which if switched off (by LSD) would result in disinhibition and a confusion of sensory modalities. In support of this hypothesis, several groups have shown an increase in the firing rate of the terminal fields of various raphé nuclei after administration of hallucinogens (Mouriz-Garcia,

1969; Horn and McKay, 1973). Of course, if some of the effects of raphé innervation of terminal fields are excitatory, then i.v. LSD, by inhibiting raphé neurones, would functionally block excitation.

One of the questions asked in this thesis was could 5-HT receptor agonist effects of phenethylamines on raphé terminal fields contribute to disinhibition (by mimicking excitatory 5-HT effects) or counteract it by inhibitory effects? LSD does not mimic excitatory 5-HT effects, but 5-MeoDMT clearly does (Bradley and Biggs, 1974; McCall and Aghajanian, 1979b). Results presented in this thesis suggest that methylated phenethylamines also can be classed with 5-MeoDMT in this respect.

Trulson et al. (1977a) provided the first evidence which questioned the importance of LSD-induced DR neurone inhibition to its mechanisms of action. This group found that at the time when a cat had developed tolerance to the behavioural effects of LSD, LSD was as effective in causing DR inhibition as it was on the first injection. Trulson and Jacobs (1979a) also reported that the behavioural effects of LSD in cats lasted longer than the period of DR inhibition. In the same study i.v. DOM did not inhibit the DR. These findings suggested that the important site for the action of LSD may be at sites postsynaptic to raphé neurones, with a longer time course of action than DR inhibition. In 1979 Rogawski and Aghajanian demonstrated that the non-hallucinogen lisuride (a close analogue of LSD) is just as potent as LSD in its ability to inhibit DR firing. Its effects on 5-HT turnover are also only quantitatively different from those of LSD

(Peri et al., 1978). Heym et al. (1984) showed that the behavioural effects of DOM and LSD were prevented by metergoline and ketanserin, but these drugs did not prevent DR inhibition caused by i.v. LSD. Taken together, this work suggests that the mechanism of action of hallucinogens is more likely to be worked out by studying postsynaptic effects.

Aghajanian has recorded from DR neurones using intracellular techniques *in vivo* and *in vitro* (Aghajanian and Vandermaelen, 1982a; Vandermaelen and Aghajanian, 1983; Aghajanian and Lakoski, 1984). These experiments revealed a pronounced spike after-hyperpolarization termed a Pacemaker Potential. This pacemaker potential shows a slow decay to threshold between each spike, which does not occur in the presence of LSD. Both 5-HT and LSD produced a decrease in DR cell input resistance, suggesting an increase in conductance. Reversal potentials for LSD and 5-HT effects suggested the hyperpolarization was caused by K^+ ion efflux.

Not all groups of raphé nuclei are sensitive to LSD or 5-MeoDMT. Heym et al. (1982) have shown that 5-MeoDMT does not inhibit neurones of the nucleus raphé pallidus. Trulson et al. (1983b) found a relationship between the direction in which the neurones projected and their response. The nucleus raphé pallidus has descending projections and its firing is not inhibited by hallucinogens. The nucleus centralis superior, however, is inhibited by indoles and LSD. Phenethylamines given i.v. had no effect on either cell group.

4. Postsynaptically mediated effects of hallucinogens

Other single cell electrophysiological studies in various brain areas provide information which does not cohere into a single hypothesis (for review see Brawley and Duffield, 1972). Lamarre et al. (1971) reported that harmaline induced rhythmic discharges in the inferior olive. Lamarre and Puil (1974) showed that microiontophoretically applied harmaline applied directly to olivary units would also induce the rhythmic multiunit activity. Headley et al. (1976) reproduced these findings and examined the role of 5-HT in causing the discharges. LSD did not induce them; also results with PCPA and putative 5-HT antagonists were inconclusive.

Unpublished results from this laboratory with methoxylated amphetamines (Parry, personal communication) are reproduced in Appendix I. DOM and PMA effects on spontaneously active superficial cerebral and cerebellar cell firing were compared with responses to D-amphetamine and 5-HT. A population of over 100 neurones was sampled to make these comparisons. In these correlation studies the overall effects of iontophoretically applied PMA and DOM resembled those of 5-HT rather than those of D-amphetamine. Where 5-HT and D-amphetamine had opposite effects, DOM was similar to 5-HT. Only the effects of PMA correlated with the direction of responses to D-amphetamine. This data provides further circumstantial evidence that the effects of DOM are directly mediated, possibly by a receptor for 5-HT, and that the effects of PMA are indirectly mediated.

McCall and Aghajanian (1980b) have reported that the responses of FMN's to NA and 5-HT were greatly increased for long time periods by intravenous or iontophoretically applied hallucinogens. Mescaline and 5-MeoDMT in large i.v. doses produced a 5-HT-like agonist effect, but i.v. lisuride did not sensitize the FMN's to either transmitter. These findings, if widely applicable, may help explain some of the peripheral effects of hallucinogens on the motor system and account for the enhancement of spinal reflexes (Andén et al., 1974).

DA agonist activity may be a factor in the mechanism of action of hallucinogens, as LSD and DOM appear to show some DA agonist activity (Pieri et al., 1974; Trulson et al., 1977b). It has been suggested (Jacobs and Trulson, 1979) that the most potent hallucinogens may be those that decrease the influence of serotonergic transmission in the brain while mimicking the actions of brain DA, however the phenthylamines appear not to inactivate brain serotonergic transmission. Clinical evidence apparently supports a role for DA in some of the effects of LSD as this drug often causes emesis in high doses; also trips are attenuated, but not stopped, by neuroleptics (Jacobs and Trulson, 1979). Finally, DA agonists (eg. apomorphine, bromocryptine and L-dopa) have been reported to have some hallucinogenic activity (Fuxe, 1979).

H. Statement of the problem

With this background, it was decided to ask the following main

questions about cells which were well defined anatomically (and not randomly encountered):

1. Was it generally true that inhibitory responses to 5-HT, at the S_3 site, could not be prevented by known 5-HT antagonists? Evidence inconsistent with such a statement had been published with regard to the inhibitory action of 5-HT on hippocampal CA_1 pyramidal cells (Segal, 1976, 1977 and 1980). Reproduction of these findings would open the way to proof that an inhibitory agonist (e.g. DOM), produced some of its effects by 5-HT (S_3) receptor agonism.
2. A controversy exists about whether DOM applied by the i.v. route has an inhibitory effect upon a ventral subgroup of DR neurones (compare Aghajanian et al., 1969, and Trulson et al., 1981). As DOM had not been tested directly upon DR cells, it was decided to compare the responses to i.v. drug with the response to direct application by microiontophoresis. By this approach, it is possible to compare the effects of DOM with those of mescaline which does not inhibit when directly applied (Haigler and Aghajanian, 1973). This experiment would provide an index of the action of phenethylamines on S_2 (or 5-HT_{1A}) receptors. The comparison with D-amphetamine, would provide an index of indirectly mediated effects (due to transmitter release).

3. Does DOM have an agonist effect on S_1 (possibly 5-HT_2) receptors on FMN's? In other words, does DOM mimic 5-HT and 5-MeoDMT in facilitating excitatory input to FMN's? The other expected action was that it may have no direct agonist effect, but instead sensitize the FMN's to 5-HT and NA. This is the nature of the effect of LSD on neurones of the facial motornucleus, where higher currents of application caused transient blockade of responses to 5-HT (McCall and Aghajanian, 1980b). If an agonist effect was present, I reasoned that it should be selectively prevented by a "D" type 5-HT antagonist, assuming the response was mediated by 5-HT receptors.

CHAPTER II

MATERIALS AND METHODS

A. Preparation

1. Experimental animals

Male Sprague Dawley albino rats (Rattus norvegicus) were purchased from the University of Alberta breeding program (Biological Sciences); the weights were in the range 180 - 220 g. A batch of five animals were stored in a cage with a sawdust floor and maintained on a 12 hr light-dark cycle at an ambient temperature maintained at 24°C. The animals had access to "Wayne rodent blox" rat chow and water ad libitum. Rats of this size were used within one week so that the usual size of the experimental animal was 240 g (usually 3 months old).

2. Anaesthesia

All experiments (with the exception of two protocols described below) were carried out under anaesthesia produced by intraperitoneally (i.p.) injected urethane. The usual dose was 1.25g/kg which produced a stable lightly anaesthetized rat with its corneal blink reflex maintained. If this dose did not produce a sufficient depth of anaesthesia, the dose was increased to 1.5g/kg. The advantage of urethane for neurophysiological experiments is its long duration of action, which negates the necessity for continual depth testing and supplementation. Some hippocampal and DR experiments were done using choral hydrate anaesthesia, to make sure that the urethane did not have unique effects. The first injection was a dose of 400 mg/kg i.p.; this had to be supplemented with 100 mg/kg i.v.

approximately every two hours. Seven experiments were carried out using halothane anaesthesia (0.8%) carried in carbogen (95% O_2, 5% CO_2) to a tracheal cannula open system. Anaesthesia compromises the ability of an animal to maintain its temperature, consequently each animal was fitted with a rectal temperature probe and placed on a heating blanket connected to a servo control and 12 V battery. This arrangement maintained the body temperature of the animal at 37°C ± 0.5°C.

3. Surgical procedures

i) Cannulation. In experiments in which drugs were given by the i.v. route the blood pressure was also monitored. The i.v. cannula was placed in the left femoral vein and another cannula was placed in the right femoral artery for blood pressure recording. Both the artery and vein were cannulated with PE10 tubing, and the arterial cannula was connected to a Statham transducer (model P23AA). The cannula was kept free from blood clots with heparin in physiological saline (200 units per ml). Skin incisions were closed with michel wound clips. The animal was then placed in a Narashige (SR5) stereotaxic apparatus. Following this, the animal's scalp was cut in the midline with a scalpel and the skin and temporal muscle were reflected laterally with a bone raspatory. At this stage some scalp muscle was crushed to facilitate blood clotting. A hole for insertion of the electrode about 2.5 mm in diameter was drilled in the skull at the appropriate location (see stereotaxic methods). After removal of the bone, the dura mater was either cut away (if it overlay

cortex) or torn in the anterior-posterior direction if it obscured the lateral sinus. The hole was then plugged with absorbable gelatin sponge (Gelfoam^{R}) which promotes clotting. After a few min the plug was removed and the wound was wiped with a cotton wool swab. The area was kept moist with physiological saline throughout the experiment.

B. Stereotaxic methods. Before the animal was put in the stereotaxic apparatus, a marker was placed at earbar zero and the lateral/medial and anterior/posterior readings of the micromanipulator noted. The rat was then placed in the stereotaxic apparatus: the earbars were placed in the external auditory meatus and gently pressed until both ear drums were perforated; the incisor bar was held in place by a clamp over the animals' snout, and was set at 0 mm elevation over the earbar zero (interaural line). The marker was then lowered and if the animal was correctly positioned the marker touched the λ reference point (the junction of the posterior skull sutures). All measurements were made from λ. After the hole in the skull was made (see Surgical Procedures), a cut was placed in both the anterior/posterior and medial/lateral planes of the sides of the hole where they were touched by the marker; these marks indicated the coordinates for electrode descent.

Hippocampal pyramidal cells in area CA_1 were found at 3.8 mm anterior to, 1.8 mm lateral to, and a depth of 2.4 - 2.6 mm ventral from the skull surface λ reference point.

DR neurones were found at 0 - 500 μm anterior to λ in the midline

at a depth of 5.8 - 6.3 mm ventral to the skull surface (see Fig. 3).

FMN's were found at 2.2 - 2.8 mm posterior to and 2.0 mm lateral to λ, at a depth of 7.5 - 8.5 mm from the brain surface (see Fig. 4).

C. Recording

1. Electrodes

(i) Manufacture

Recording was found to be most satisfactory when the recording barrel was made of thick-walled borosilicate glass (Hillgenberg, O.D. 2mm, I.D. 1 mm). This glass is preloaded with a glass fibre to facilitate barrel filling by capilliarity. Drug barrels were made out of thinner walled glass (O.D. 1 mm) and also were preloaded with glass fibre. This arrangement served to keep the barrel array to a manageable size and bias the centre barrel towards a low resistance.

The five-barreled electrode blanks were made in a cross configuration (in order to reduce cross-talk and salt bridge formation) with the recording barrel in the centre. This was achieved by placing a 5-6 cm piece of the recording barrel glass flat on a bench with one piece of the drug barrel glass at either side of it. These pieces were glued at each end using a small amount of quick setting epoxy resin glue. A fourth barrel was placed on top of the centre recording barrel and held in place with modeling clay until the glue set. The next stage involved turning the array over, and the

addition of one more barrel onto the recording barrel. After trimming off excess glue, a 3 mm wide ring of shrink tubing was placed on each end. The last stage in the manufacture of the blanks involved winding 2 pieces of wire alloy around the centre of the electrode, about 3 cm apart. This kept the barrels together during pulling. The blanks were clamped into a Narishige PE2 electrode puller.

The centre of the blank was heated and twisted at least 360° whilst being allowed to drop 3-5 mm. The heat was then turned off and the magnet and heating wire adjusted for the final pulling of the electrode. Finished electrodes were broken back under visual control, using a brass bar mounted on a micromanipulator to a tip diameter of 10-15 µm. The barrels were marked with a waterproof colour pen and filled using a 5 cm 32-gauge needle attached to a syringe. When the barrels were filled with the appropriate electrolyte, the openings were sealed with silicone grease. This procedure served to prevent the solution from evaporating and the formation of salt bridges. The centre recording barrel contained 2% pontamine sky-blue dye and 0.5 M sodium acetate and commonly had a resistance of 4-8 MΩ. In most experiments, one barrel was assigned to current balancing and was filled with 0.15 M NaCl.

2. Equipment

The centre barrel of the micropipette was connected by a platinum alloy wire to a field effect transducer (Dagan) and used to record

single unit spikes extracellularly. The output was fed to an A.C. preamplifier with a gain of 1000 (Dagan model 2400Z). This output stage was coupled, by way of a 1μF capacitor, to a 3A9 differential amplifier (Tektronix) of a dual beam cathode ray oscilloscope. The output of the oscilloscope amplifier stage was fed into a variable window discriminator; the output from this was then integrated and counted with a rate meter (Ferch electronics). The frequency of extracellular unit activity was then traced out on one channel of a Beckman Type RP dynograph with a rectilinear pen system. Extracellular potentials were also monitored with a loudspeaker incorporated in the window discriminator, so that it was not necessary to view the oscilloscope continuously for spikes. To aid in photography of extracellular potentials, one output of the window discriminator was also fed to the z-axis of the oscilloscope to intensify the beam during the falling phase of the action potentials. A block diagram of the experimental arrangement is shown in Fig. 2. The equipment used is listed in Table 3.

D. Physiological identification of cells

In the hippocampus, as elsewhere, extracellular action potentials were viewed on an oscilloscope and single cells discriminated by spike height. Most cells encountered in urethane-anaesthetized rats were slow firing or silent; the latter were induced to fire with a low current of ACh or GLU. During the experiments, a small current of ACh or GLU was used to maintain a stable firing rate of 10-20 SPS. This also allowed for a more consistent assessment of inhibitory

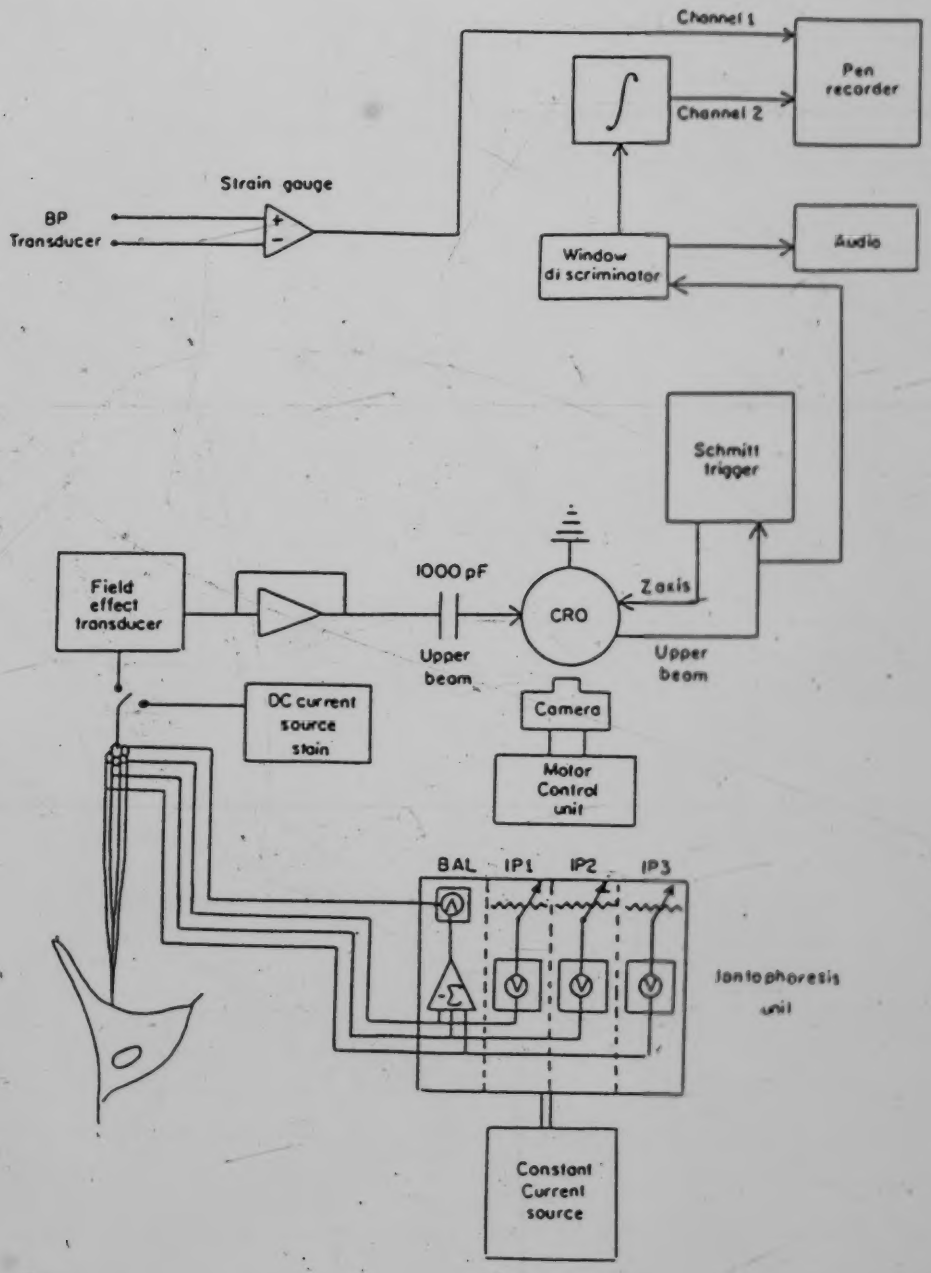


Fig. 2 Diagram of the experimental arrangement used for extracellular recording and drug iontophoresis.

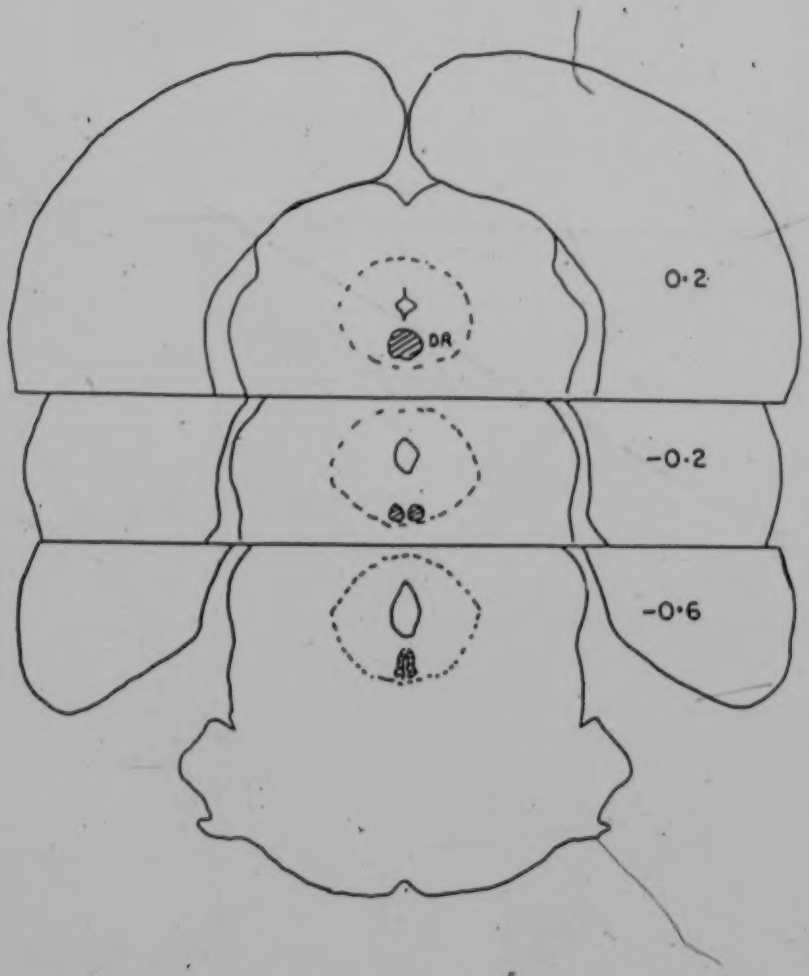


Fig. 3 Diagrammatic representation of coronal slices through the rat brain at the level of the pons. Numbers on the right are millimeters from λ. The shaded areas represent the location of the dorsal raphé nucleus.

Figure adapted from the atlas of Pellegrino et al. (1981).

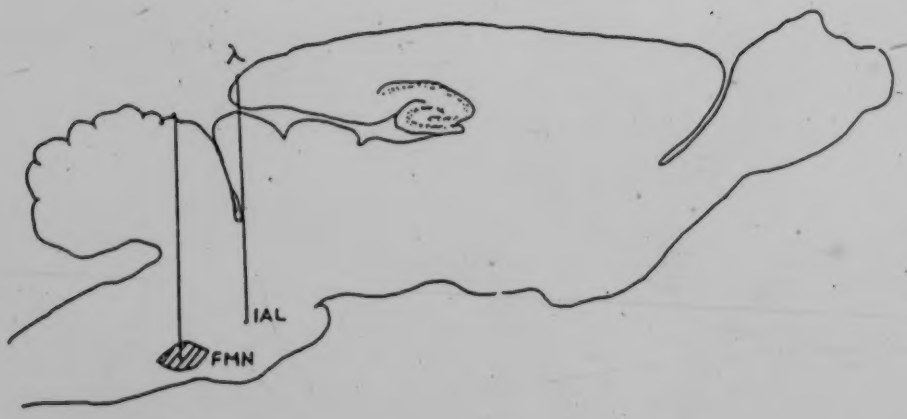


Fig. 4 Diagrammatic representation of a sagittal plane slice through a rat brain at approximately 2 mm lateral to the midline. The approximate relationship between the positions of λ, the interaural line, and the facial motor nucleus are shown. Figure adapted from the atlas of Pellegrino et al. (1981).

drug effects between experiments. CA_1 pyramidal cells fire with a characteristic pattern of bursts; each burst consists of several large spikes with a gradual reduction in extracellularly recorded action potential size from the first to the last spike. This pattern often alternates with single spikes (Kandel and Spencer, 1961).

DR cells were identified by their slow (1-2 SPS) regular firing rate, and by their wide action potentials (1-2 msec) with the presence of a "notch" in its upward sweep (Aghajanian et al., 1969). If the cell was inhibited by a small current of 5-HT, the cell was studied further.

FMN's were located by ejecting GLU (20nA) until a large action potential was recorded (often more than 1 mV peak-to-peak with a 8 MΩ electrode and a noise level of approximately 100 μV); such cells would fire usually at or above 15 SPS. The GLU current was then turned down to about 8 nA (or approximately 1/3 of firing threshold) and a cycle of 5-HT application started (usually 20 nA for 30 sec every 99 sec). With this protocol the cells were silent, but were excited to a firing rate of 10-15 SPS near the end of the 5-HT application (McCall and Aghajanian, 1979). This excitation would often continue for 2 or 3 times the duration of the application of the 5-HT.

E. Drugs and Solutions

Most drugs were made up in double-distilled deionized water and

adjusted to an optimum pH for ionization (see Table 2). An effort was made not to deviate from the pH 4-8 range. For example, pH 8 rather than 7.2, was used for GLU so that the drug is predominantly in the anionic form. In the case of ketanserin, pH 4 rather than 6 was used, as above pH 4 the drug would precipitate. All solutions were filtered through 0.22 μm type 'GS Millipore' paper to remove particulate matter. The drugs and solutions used are listed in table 2.

Table 2

Drugs and solutions:

Acetylcholine Cl (Sigma, 0.5 M, pH 5)

D-amphetamine sulphate (SK & F, 0.1 M pH 5)

Chloral hydrate (0.5g/ml in H_2O)

Chlorpromazine HCl (Sigma, 50 mM pH5 in 0.15 M NaCl)

Cinanserin HCL (Nutr. Biochem/S. Corp., 50 mM pH 4)

Cyproheptadine HCl (Merck, 5 mM pH 4)

(+)-2,5-Dimethoxy-4-methylamphetamine HCl (Natl. Bureau Drug. Res., 0.1 M pH5 with and without 0.15 M NaCl)

Dopamine HCl (Sigma, 0.1 M pH 5)

Ethyl carbamate (Urethane, Pfaltz & Bauer, 500 mg/ml in H_2O)

Formalin phosphate (Fisher Scientific, 10% buffered)

Glutamate monosodium salt (MC & B chemicals, 0.1 M pH 7.8)

Ketanserin tartrate (Janssen, 20 mM pH 4)

Lysergic acid diethylamide (Sandoz, 1 mM in 0.15 NaCl pH 5)

MDL 72222 Methane sulphonate (Merrell Dow, 0.02 M pH 4)

Mescaline HCl (R. Coutts, 0.1 M sometimes in NaCl pH 5)

Metergoline (Farmitalia, 20 mM in 75 mM Ascorbic acid pH 4)

2-Methylbutane (Fisher Scientific)

Methysergide bimaleate (Sandoz, 8.5 mM in 0.15 M NaCl pH 5)

L-Noradrenaline bitartrate (Winthrop, 0.1 M pH 4)

Phenoxybenzamine HCl (SK & F, 5.7 mM in 0.15 M NaCl pH 3)

Phentolamine HCl (Ciba, 50 mM pH 5)

Picrotoxin (Sigma, 10 mM in 0.15 M NaCl pH 5)

Pontamine Sky-blue dye (2% in 0.5 M Na acetate pH 8)

Serotonin bimaleate (Koch-Light, 0.1 M pH 4.5)

Serotonin creatinine SO_4 (Sigma, 40 mM pH 4.5)

Sotalol HCl (Bristol Myers, 0.1 M pH 4)

Trifluoperazine HCl (SK & F, 50 mM in equimolar tartaric acid pH 4)

F. Data display

1. Photography

When local anaesthetic effects (suggested by a reduction in spike amplitude) or increases in spike size caused by a substance ejected by microiontophoresis were observed, then photographic records were taken. This was done with a Shakman AC 2/25 Oscilloscope Camera by moving the film at 0.5 to 2 cm/sec whilst keeping the position of the beam constant. Additional photographs of single spikes were taken with a Tektronix 'Polaroid' C30A camera.

2. Data analysis and statistics

(i) Hippocampal recording.

The early hippocampal experiments were designed to re-evaluate the report of the antagonism of inhibitory responses to 5-HT (Segal, 1976). As others had found, the putative antagonists suppressed firing. This presented a difficulty when it came to measuring the responses. Clearly one can measure the magnitude of the 5-HT-induced inhibition in relation to the original (control) level of firing, or to the firing level at the time of the agonist application (which would be reduced during the presence of the "antagonist". Stated in other words the problem is one of whether to measure the net inhibition caused by the agonist, or the total inhibition produced by the antagonist plus agonist. The first of these choices is inappropriate (if there is a possibility that the putative antagonist may be a partial agonist), since strong suppression of firing by the

putative antagonist could partly occlude the inhibition by the agonist, even in the absence of antagonism. Thus it is reasonable to report inhibition during suppressed firing by the antagonist as total inhibition from the original control as a percentage of total inhibition possible from control, rather than to simply relate the absolute agonist effect to the magnitude of the control percent inhibition (Ariens et al., 1964). In order to compare the two methods of measurement, the results are reported using both methods, both as the total inhibition (in terms of the original baseline) and as the net inhibition.

Only trials where at least some decrease in baseline firing occurred (as evidence of antagonist ejection) were included in the statistical analysis. Responses to 5-HT were expressed as a percent of the baseline firing rate; usually an average of three responses during antagonist ejection were compared to the average of three responses of the immediately preceding controls by paired t test (n = number of cells). This approach was taken to measure the interaction between other agonist/antagonist drugs in the hippocampus.

(ii) The Dorsal Raphé

In order to assess the ability of four compounds to inhibit the spontaneous firing of DR cells, responses with increasing currents were obtained until either the cell was completely inhibited, or a non-specific local anaesthetic effect occurred. In other words, a maximal degree of inhibition possible was found. When inhibition of

greater than 50% was seen, an $I.T._{50}$ value was calculated according to the method of de Montigny and Aghajanian (1977). Briefly, the method involves recording from a regularly firing neurone. An inhibition of firing rate to 50% of control is obtained by the drug ejection; this must occur within 10-50 sec as during this time drug release increases linearly (Simmonds, 1974). The time to 50% firing rate is multiplied by the current in nA, to give an $I.T._{50}$ value (in coulombs). In two cells of the same type (provided one is using the same electrode) the $I.T._{50}$ values represent equieffective doses of drug (these values produce the same response). If in each animal the $I.T._{50}$ is evaluated several times on each cell, then the geometric mean (antilog of the mean log) of the $I.T._{50}$'s is normally distributed. This is because equieffective doses of an agonist are normally distributed on a logarithmic scale rather than an arithmetic one (Fleming, 1972). In addition, the maximum inhibition of baseline firing rate was obtained for each cell, averaged for each drug, and quoted as mean $\pm$ standard error of the mean (SEM).

(iii) Facial Motor Neurones

Drugs were applied in regular cycles for a period of 30 sec, followed by 99 sec pause. Regular responses to alternating applications of the test and control agonists were obtained for a 12 min control period before the antagonist was added; after antagonist application this cycle was then repeated for as long as possible. Results were measured as the average size of three excitatory

excitatory responses in the control (assigned 100%), antagonist, and recovery periods. Data was pooled and averaged for each antagonist applied (usually the specificity of the antagonism was also assessed).

Excitatory responses in the different conditions were expressed as mean ± SEM.

G. Histology

1. Method for site marking. The recording electrode contained 2% pontamine sky-blue dye and 0.5 M sodium acetate. The dye is negatively charged at pH 8 and was ejected by the passage of negative current (10 μA) from the recording electrode. This was done for 10 min at the end of each experiment according to the method of Hashimoto and Kaneko (1967).

2. Fixation

The animal was then perfused with 10% buffered formaldehyde solution by placing a needle (connected by rubber tubing to a gravity feed reservoir of formaldehyde) into the rat's heart. After fixation, the brain was excised and stored in formaldehyde.

The brain was then trimmed around the marked site, mounted on tragacanth gum and covered with glycerol. The sample was then placed in 2-methylbutane (which had been cooled in liquid nitrogen) for 15 sec; this resulted in a block of tissue which was frozen solid.

The block was mounted in a 'Slee' freezing microtome. 50μm sections were cut and sections containing blue dye-spots were placed on microscope slides.

3. Staining. The sections were allowed to dry, and 'Permount' fixative was applied around the edges of the sections to fix them to the slide; just before staining the sections were rehydrated. The sections were then exposed to 2.5% safranin dye for 2 min before the excess was washed off. Safranin is a basic cationic dye which stains anionic groups; it is, amongst other things a nuclear stain. Safranin's red colour contrasted well with the blue of pontamine sky blue. After staining, the sections were put through a succession of ethanol baths each of higher concentration: 70%, 90%, 95% and 98%. This process was adjusted to allow destaining to the correct degree. Finally the sections were placed in a solution of 98% ethanol/xylene (1:1, v/v), followed by a xylene bath to clear the slide. The slides were treated with 'Permount' and a coverslip placed over them. Within 30 min they were ready to be viewed under the microscope.

Table 3

Equipment Used

Oscilloscope Camera Shackman AC 2/25

Beckman Type RP Dynograph

Strain Gage Coupler Type 9853

Preamplifier Type 481B

Dynograph Amplifier Type 482A

Power Supply Type 382C

Dagan Preamplifier 2400Z Extracellular Current Pump

Tektronix Dual Beam Oscilloscope Type RM 565

Medical Systems Corp Iontophoresis Unit Type MS-2B

Neurophore BH2 & Power Supply

Narishige Stereotaxic Apparatus SR-5

& Micromanipulator

Burleigh Inchworm Controller Type PZ 550

Honeywell Digital Meter Model 333R

Lambda Power Supply Model LPD-423AFM

Foregger Anaesthetic Machine Model 100

Statham Pressure Transducer Model P23AA

Ferch Gate Descriminator

Ferch Rate Meter

Temp Control Unit (Technical Services U of A)

Dayton Dental Drill Model 2M033

Slee Freezing Microtome Type HRM

Narishige Electrode Puller Type PE-2

Research Industries Power Supply No. 122-64000

(Output 110-120V DC)

Mettler Balance

CHAPTER III

RESULTS

The objective of the first section of this study was to determine the receptor dependence of any effects that DOM may have upon the firing of hippocampal CA_1 pyramidal cells. Studies discussed in the introductory chapter of this thesis present findings which point to a central 5-HT receptor agonist effect of DOM as its most prominent action. For this reason, before the effects of DOM in the hippocampus were studied, the response of CA_1 pyramidal cells to 5-HT was investigated. Initially a re-evaluation of a report of the successful antagonism of the response of these cells to 5-HT (Segal, 1976, 1980) was undertaken. It was reasoned that if this finding could be reproduced and the selectivity of the antagonism assessed, then the same procedure carried out for DOM may lead to the elucidation of the receptor types involved in its action.

A. Recordings from hippocampal CA_1 pyramidal cells

1. Effects of acetylcholine and glutamate

Stable long-lasting recordings were made from 147 hippocampal pyramidal cells; histological examination of the recording sites confirmed their location to be area CA_1. As described in the methods (Section D), the CA_1 cells were silent in the urethane-anaesthetized rat; a small continual ejection of ACh (1-10 nA) caused the cells to fire action potentials, slowly at first, but within 1 or 2 min a stable firing rate of about 10 SPS was reached. The activation of CA_1 cell firing by GLU had a rather different time course. Similar application of GLU currents produced an activation of the cell which

observed within 30 sec, and had often reached its maximal rate by this time. GLU-elicited firing was not usually as regular or as well maintained over time as the ACh-maintained firing. These observations are in keeping with those reported by Biscoe and Straughan (1966).

2. The action of iontophoretically applied 5-HT

Of all the cells studied in urethane-anaesthetized animals, only two neurones were unresponsive to a short pulse of 5-HT (5-25 nA for 10 or 15 sec) which normally produced a brief inhibition of cell firing. The most likely explanation for the failure of 5-HT to inhibit these cells would invoke technical problems associated with the ejection of current from the electrode. In the halothane-anaesthetized rat, 4 spontaneously firing cells were encountered; their firing was also inhibited by a small ejection of 5-HT. 55% of all cells showed a period of rebound-excitation which followed the brief inhibition caused by 5-HT. This excitation was only noted if the firing during this period was faster than control rates. Rebound-excitation usually lasted for 10-20 sec (See Figs. 6b and 10a). Rebound-excitation to 5-HT application was also noted by Segal (1976). Excitation which was not preceded by a period of inhibition was never observed. Similar and higher currents from electrodes containing NaCl (0.1 M) at pH 4 did not affect cell firing rates. Results from a number of experiments also showed that continuous 5-HT application had an inhibitory action upon firing elicited by short

pulses of both ACh and GLU.

No signs of desensitization of the response to 5-HT was observed, despite attempts to desensitize the putative receptor. Low currents of 5-HT (3 nA) were applied during responses to 5-HT, and the responses to 5-HT remained unchanged (Fig. 9a). It is noteworthy that the final level of inhibition reached by the application of 5-HT pulses was not increased simply by reduction by 5-HT of the overall control firing rate. Larger pulses of 5-HT (Fig. 9b) for periods up to 2 min seemed to produce a proportionately longer period of inhibition, but no clear evidence of desensitization of the response to 5-HT was discernable. On 4 cells (in 4 animals) i.v. injection of the 5-HT-selective uptake blocker fluoxetine (1 mg/kg given cumulatively to 3 mg/kg) did not alter the magnitude or the duration of the response to 5-HT.

3. The action of classical 'D' type 5-HT antagonists upon CA_1 cells and their response to 5-HT

Segal (1976), on the basis of results obtained in vivo reported that methiothepin, mianserin and cinanserin only suppressed hippocampal CA_1 cell firing and did not antagonize inhibition caused by 5-HT. However he also reported that methysergide, cyproheptadine and LSD could antagonize 5-HT. For this reason, the last three compounds were studied further. In addition, the effects of metergoline, ketanserin (the 5-HT_2 antagonist) and the 'M' receptor antagonist MDL 72222 were examined. Metergoline had been reported to

antagonize, with a certain degree of selectivity, the inhibitory action of 5-HT on cortical cells of the rat (Sastry and Phillis, 1977b).

(i) Urethane-anaesthetized animals

Methysergide was applied a total of 17 times (trials) to 9 cells in 5 animals; in 7 trials the cell firing was maintained by GLU (Fig. 6a) and in 10 trials by ACh (Fig. 6b). Methysergide did not antagonize the effects of a submaximal application of 5-HT. Even doses of methysergide sufficient to cause suppression of pyramidal cell firing did not prevent the effects of 5-HT (Fig. 6a, 6b; Tables 4 and 5). On several occasions methysergide caused a local anaesthetic type effect (indicated by the depression of spike amplitude (Curtis, 1968)) and data from these trials was excluded from the analysis. The failure of methysergide to antagonize the effects of 5-HT was also observed in 3 other experiments from 3 cells which were firing spontaneously. Data from these cells was not included in the statistical analysis reported in Tables 4 and 5 because only maximal or near maximal inhibitions in response to 5-HT could be obtained.

Cyproheptadine was applied to 11 pyramidal cells, in 5 animals. GLU application was employed to maintain firing in 10 trials (Fig. 7b) and ACh was used in 5 others. In all cases cyproheptadine did not change the inhibition caused by 5-HT even when the control baseline firing rate was reduced (Table 4), and the total inhibition produced was not altered (Table 5). No current of cyproheptadine used caused a depression in spike amplitude (ie. no local anaesthetic effect was seen).

TABLE 4. Difference in percent inhibition caused by 5-HT "antagonists"

"Antagonist"	Trials	Cells	C-T%	SE	t	P
Methysergide	17	9	+3.6	1.77	2.03	NS
Cyproheptadine	15	11	-2.81	3.08	0.91	NS
Metergoline	8	4	+0.50	5.20	0.09	NS
Ketanserin	9	5	+6.60	2.56	2.58	NS
LSD	14	6	-3.50	2.97	1.18	NS
Methysergide*	23	15	-1.33	2.62	0.51	NS

*Halothane anaesthetized animals

Inhibitions were measured as a percent of the baseline at the start of each inhibitory response. All results were obtained in urethane-anesthetized rats, except where it is indicated that halothane was used. Spontaneously active cells were included only in the halothane data, and only trials where the antagonist dose was sufficient to depress firing are included. Inhibition is reduced if the value of C(ontrol)-T(est) is positive. Significance is by "paired t", comparing the inhibition during antagonist with the immediately preceding inhibition ("n" equals the number of cells).

TABLE 5. Difference in total inhibition caused by both 5-HT and the "antagonist"

"Antagonist"	Trials	Cells	C-T%	SE	t	P
Methysergide	17	9	+0.11	1.75	0.06	NS
Cyproheptadine	15	11	-4.90	2.70	1.81	NS
Metergoline	8	4	-1	4.81	0.21	NS
Ketanserin	9	5	-3.0	1.26	2.38	NS
LSD	14	6	-5.0	2.63	1.90	NS
Methysergide*	23	15	-5.6	2.07	2.70	<0.02

*Halothane anaesthetized animals

Inhibitions are measured from the original baseline firing during control inhibitions, and are the sum of reductions in firing due to BOTH antagonist and 5-HT. This is the appropriate method of estimating antagonism by partial agonists or antagonists (Ariens et al., 1964). Other details are as in Table 4.

Metergoline was applied to 4 cells in 4 animals. GLU was used to maintain firing in 2 trials and ACh was used in 6 (Fig. 7a). As seen with the other antagonists, the effect of submaximal doses of 5-HT were not reduced by doses of metergoline which did not alter the control baseline firing rate. Higher doses caused a suppression of firing rate (not action potential size) without reducing the response to 5-HT (Tables 4 and 5).

In 4 animals ketanserin proved to have identical effects to the other drugs tested (9 trials in 5 cells). Large currents of ketanserin suppressed firing (Fig. 8a), but neither large nor small doses interfered with the inhibition produced by 5-HT (Tables 4 and 5). As with the other compounds, the development of the inhibition appeared to be neither delayed nor slowed (Simmonds, 1974).

It was also noted that each of the putative antagonists reduced the size of the rebound-excitation which occurred after 5-HT (Figs. 6b and 7b) in 85% of the instances where rebound-excitation was observed.

LSD was applied to a total of 6 cells (firing was maintained by ACh) in 4 animals. The magnitude of the average response to 5-HT was slightly greater in the presence of LSD, although not significantly so, by either method of measurement (Tables 4 and 5).

(ii) Halothane-anaesthetized animals

Because of differences between our results and those of Segal

(1976), halothane anaesthesia was used for 7 animals to duplicate exactly the conditions of the former experiments. Methysergide was applied 23 times to a total of 15 cells. In 16 of these trials ACh was ejected from the electrode to maintain firing, while the remaining 7 trials were carried out on spontaneously firing cells. Results in both situations were virtually identical to results in urethane-anaesthetized animals (Fig. 6c). When the responses to 5-HT in the presence of methysergide were measured relative to the original control baseline firing, methysergide actually appeared to potentiate the 5-HT-induced inhibitions (Fig. 6c and Table 5). There was no discernable antagonism when the responses were measured from the baseline firing level at the start of each response (Table 4).

4. The action of DOM and miscellaneous compounds previously suggested to interact with 5-HT receptors

Phenoxybenzamine is an irreversible receptor alkylating agent, which will alkylate the peripheral smooth muscle "D" receptor (Gaddum and Picarelli, 1957), albeit at higher concentrations than those required to alkylate the α_1-adrenoceptor. In 5 animals (5 cells) the application of even large currents of phenoxybenzamine (Fig. 5b) failed to prevent or even reduce the average size of the 5-HT-induced inhibition, although the baseline control level of firing was often severely but reversibly depressed.

MDL 72222 is a tropine ester of cocaine which is a potent antagonist of the actions of 5-HT on peripheral nerve "M" receptors

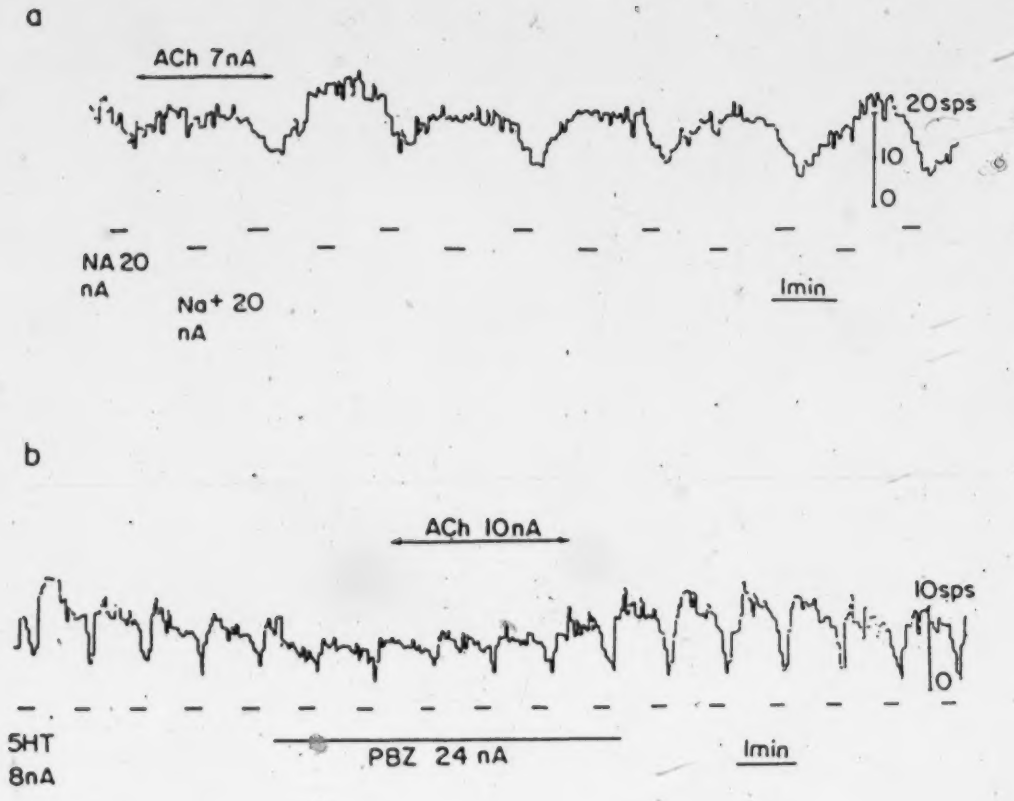


Fig. 3 Effect of NA and 5-HT on hippocampal CA_1 cells in vivo.

(a) Firing of a single hippocampal CA_1 pyramidal cell is inhibited by the iontophoretic application of NA. Note that the response is slower in onset and offset than is the response to 5-HT (below), also that the same current of Na^+ ions (at the same pH) has no effect upon CA_1 cell firing.

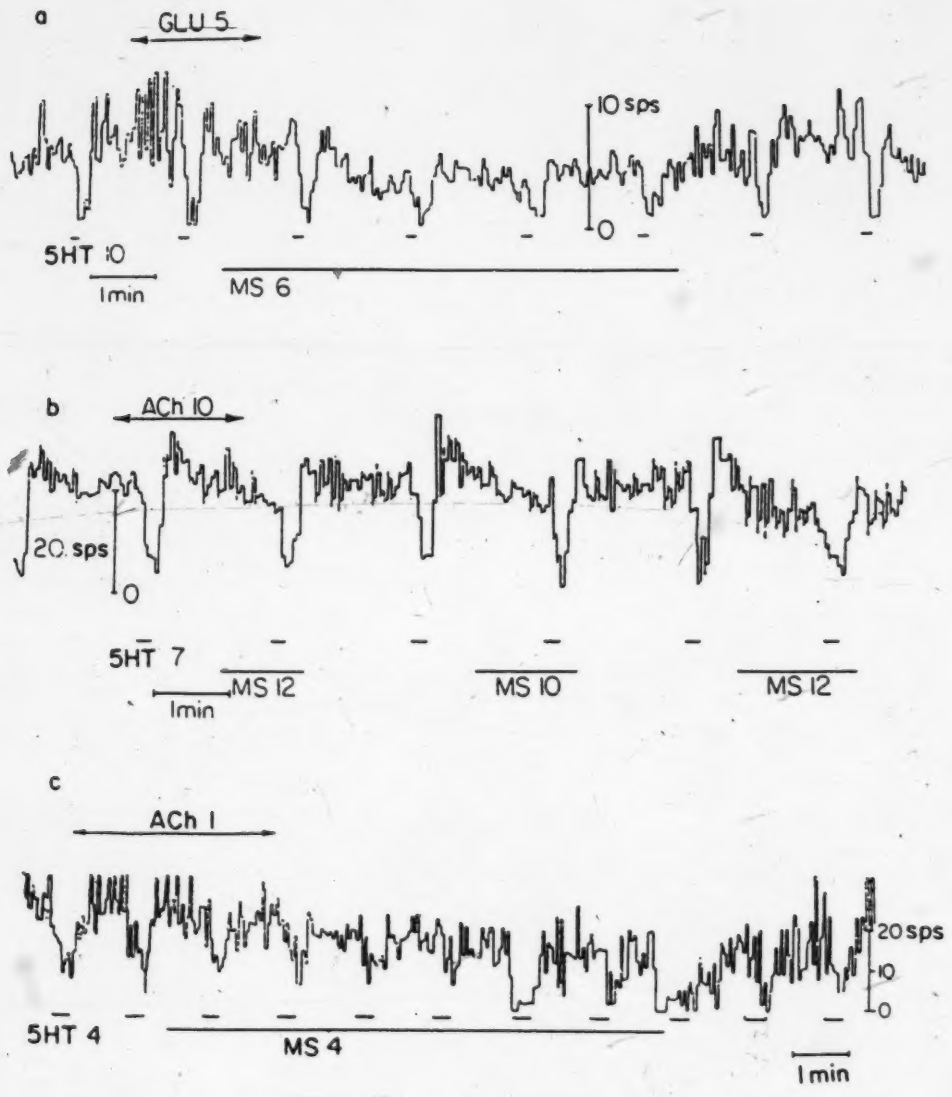
(b) Inhibitory responses of a CA_1 cell to 5-HT Phenoxybenzamine (PBZ) application did not antagonise these responses (n = 5 cells). SPS is short for spikes per second.

Fig. 6 Effect of iontophoretically applied methysergide (MS) on hippocampal CA_1 neurone firing and responses to 5-HT. Single cell firing was maintained by either glutamate (GLU) or acetylcholine (ACh), as indicated by the double-headed arrows above each record. The top two records were obtained in urethane-anaesthetized rats and the bottom one in a halothane-anaesthetized rat.

(a) A low dose of MS applied for an extended time period gradually reduced the neurone firing rate; however the final firing rate reached as a result of 5-HT application was not altered by MS.

(b) Higher doses of MS applied for briefer periods slightly reduced firing rate, blocked after excitation, but did not reduce inhibitory responses to 5-HT.

(c) Record from a halothane-anaesthetized rat. Firing was maintained by a small ACh current. Although MS decreased the firing rate, no antagonism of 5-HT-induced inhibition occurred.


a
GLU 5
10 sps
0
5HT 10
1min
MS 6
b
ACh 10
20 sps
0
5HT 7
MS 12
1min
MS 10
MS 12
c
ACh 1
20 sps
10
0
5HT 4
MS 4
1 min

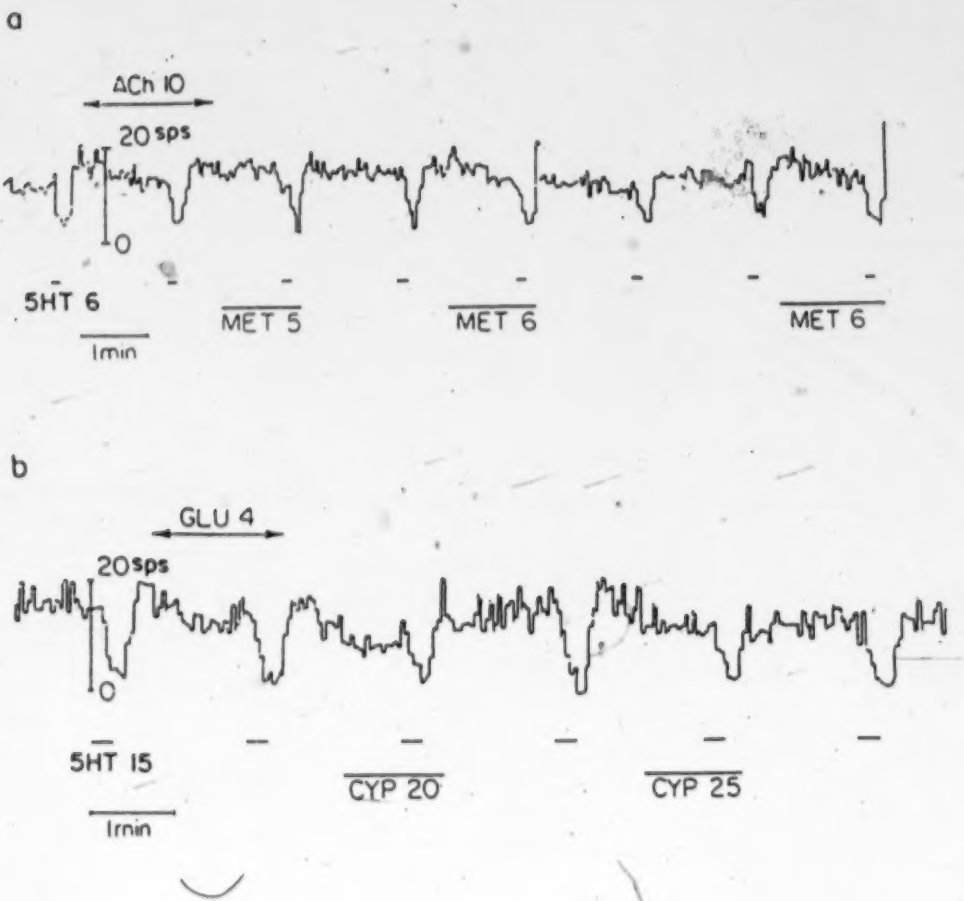


Fig. 7 Hippocampal CA_1 neurone firing and the effect of metergoline and cyproheptadine on responses to 5-HT. (a) Metergoline (MET) caused a slight reduction in the ACh-maintained firing of this cell without altering 5-HT-induced inhibition. This cell did not exhibit re-bound excitation after the 5-HT application.

(b) Cyproheptadine (CYP) reduced firing rate and after-excitation, but did not reduce the inhibitory response to 5-HT.

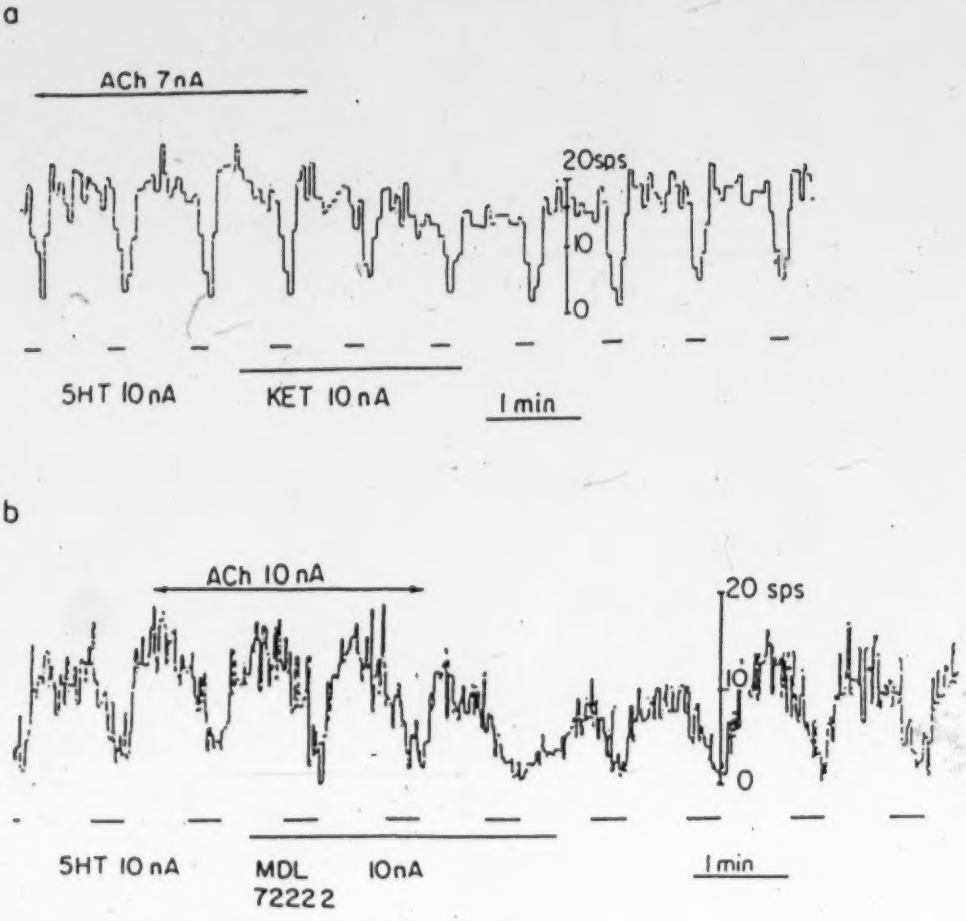


Fig. 8 Hippocampal CA_1 neurone firing; responses to 5-HT and effects of ketanserin and MDL 72222.

(a) Ketaserin (KET) caused a reduction in the ACh-maintained firing of this cell without altering 5-HT-induced inhibition.

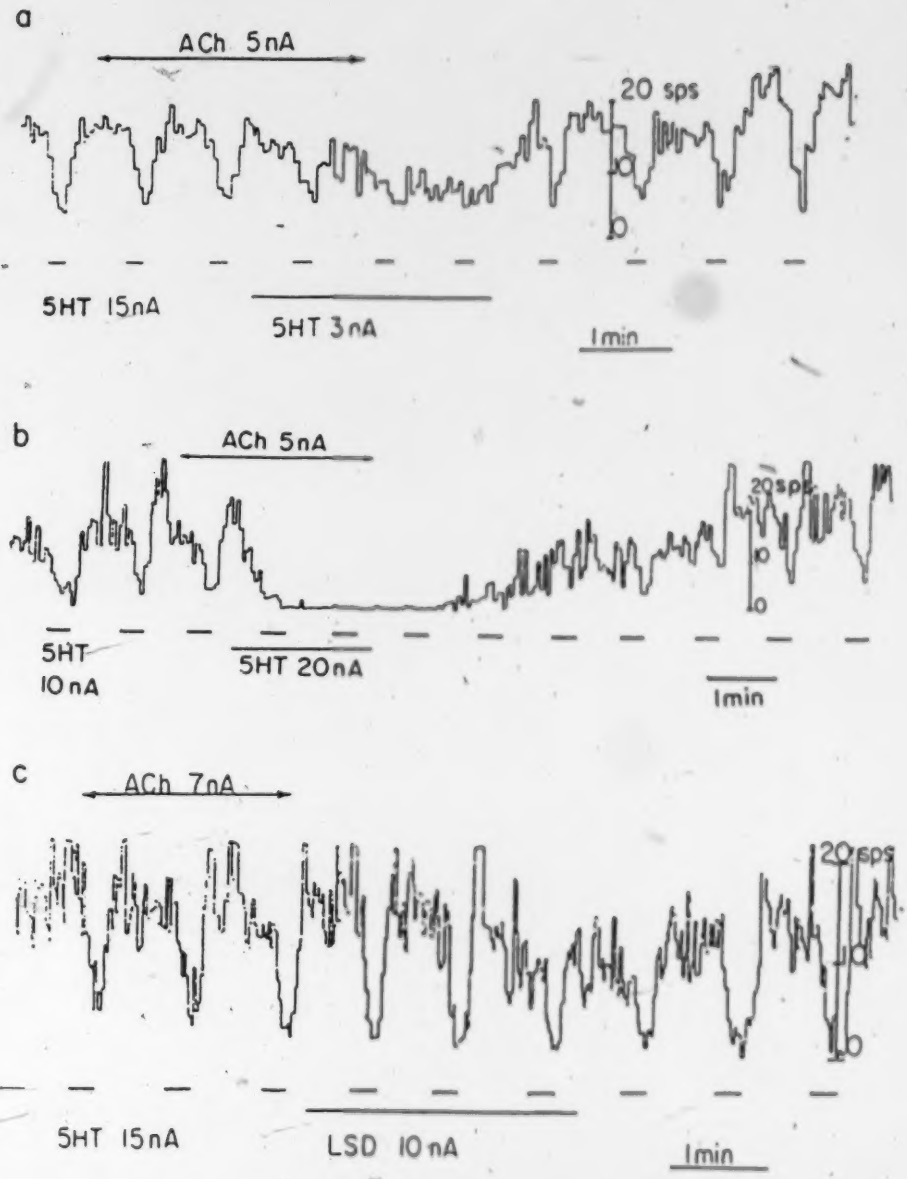
(b) MDL 72222 did not interfere with the inhibitory potency of the 5-HT pulses, even though the ACh-maintained firing rate was reduced about 50% by the drug (n = 3 cells).

Fig. 9 Attempts to desensitize the response of CA_1 pyramidal cells to 5-HT.

(a) The application of 5-HT at a low current for a prolonged period of time to a firing CA_1 neurone did not interfere with (desensitize) the inhibitory effectiveness of the pulses of 5-HT. Nor did the final level of inhibition in response to 5-HT appear to increase as a result of basal firing rate inhibition (n = 2 cells).

(b) Higher currents of 5-HT than those applied in Fig. 7 (a) did not desensitize the response of this hippocampal CA_1 pyramidal cell to 5-HT.

(c) LSD application decreased the firing rate of this CA_1 neurone in a reversible fashion, but no reduction in the size of 5-HT induced inhibitory responses could be observed.


a
ACh 5nA
20 sps
5HT 15nA
5HT 3nA
1min
b
ACh 5nA
20sps
5HT
10nA
5HT 20nA
1min
c
ACh 7nA
20sps
5HT 15nA
LSD 10nA
1min

(Fozard, 1984). This compound was tested on the response of hippocampal CA_1 cells to 5-HT in 3 animals (3 cells). Figure 8(b) shows that MDL 72222 was quite effective at reducing the control baseline firing rate, but it did not reduce the inhibitory effect of 5-HT on cell firing rate.

The effect of iontophoretically applied DOM on ACh-maintained CA_1 cell firing is illustrated in Figs. 10(a) and (b) and on responses to pulsed application of ACh and GLU in Fig. 10(b). Similar traces to those shown in Fig. 10 were obtained in 8 animals (8 cells). 5-HT application produced its characteristic (short time course) inhibition, but application of a larger current of DOM did not inhibit to the same degree. The inhibition that did occur after DOM application was much longer lasting than that seen in response to 5-HT. It is also clear from Fig. 10(a) that this persisting inhibition did not carry over to reduce the prominant rebound-excitation following responses to 5-HT. This lack of an interaction with responses to 5-HT was also seen when low currents of DOM were applied for prolonged time periods (Fig. 10b).

Responses to DOM consistent with those seen in the upper two traces of Fig. 10 were obtained when DOM was applied during pulses of either ACh or GLU. ACh had to be applied at a higher current for twice the time period than was necessary for GLU, in order to obtain a similar degree of excitation (note the time scale is different in the two traces). Firing in response to GLU occurred almost immediately,

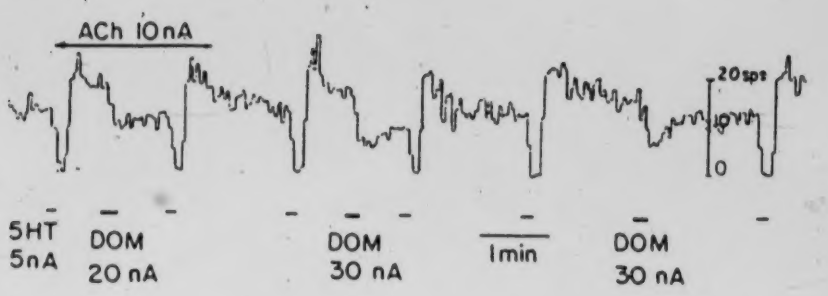
Fig. 10 The effect of DOM on CA_1 firing and its interaction with some neurotransmitter candidates.

(a) Illustrates the finding that high currents of DOM delivered in short pulses did not interfere with 5-HT-elicited inhibitions of CA_1 (single cell) neuronal firing. This cell showed pronounced after excitations following pulses of 5-HT which were not prevented by the application of DOM.

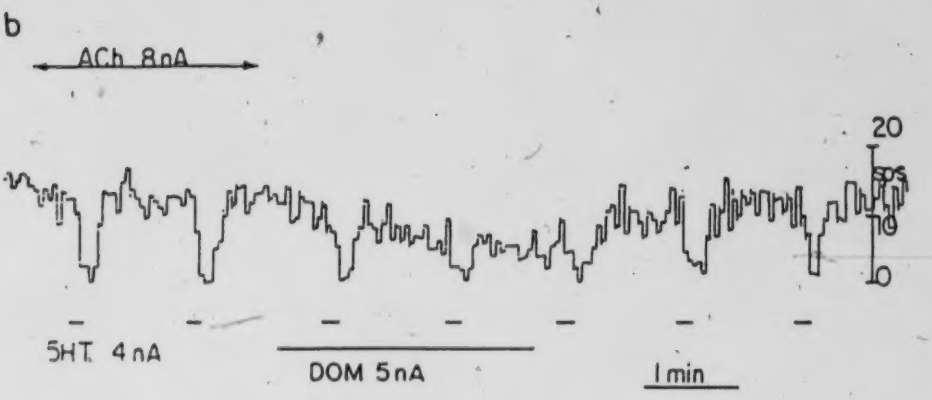
(b) Similarly the application of DOM with a low current for a prolonged period of time failed to interfere with the inhibitory effectiveness of the 5-HT pulses (n = 8 cells).

(c) Shows that DOM application will reduce the excitatory effect of pulses of ACh and GLU on single CA_1 pyramidal cells.

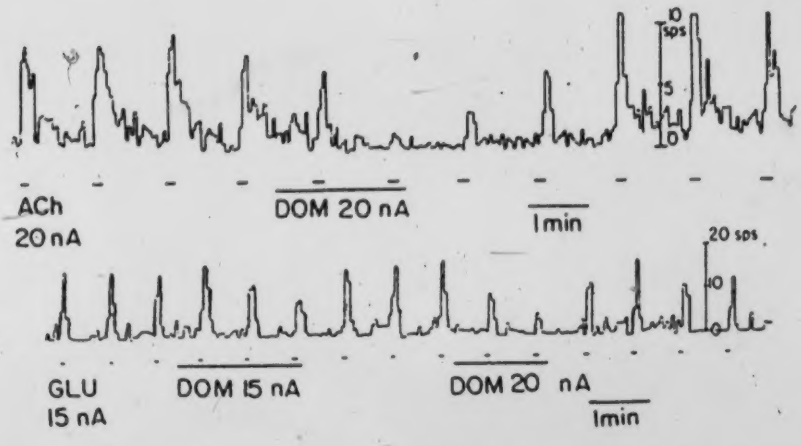
a


ACh 10nA
20sps
10
0
5HT
5nA
DOM
20 nA
DOM
30 nA
1min
DOM
30 nA

b


ACh 8nA
20
sps
10
0
5HT 4nA
DOM 5nA
1min

c


10
sps
5
0
ACh
20nA
DOM 20 nA
1min
20 sps
10
GLU
15 nA
DOM 15 nA
DOM 20 nA
1min

and ceased immediately the current was terminated, but ACh-induced firing took 20-30 sec to reach its maximum and just as long to decrease after the current was turned off. DOM decreased the response to both ACh and GLU in a reversible fashion. In every case where DOM was applied to CA_1 cells, there was no effect noted upon the size or shape of ACh or GLU-elicited action potentials. Where ACh and GLU pulses were alternated, both NA and 5-HT applied continuously reduced the response of the cell to the putative excitatory transmitters. 5-HT or NA application did not appear to reduce differentially the response of the cells to GLU or ACh pulses in a reproducible fashion (n=5 cells). Thus it was not possible to show that DOM interfered preferentially with ACh or GLU-mediated responses in a way common to either NA or 5-HT.

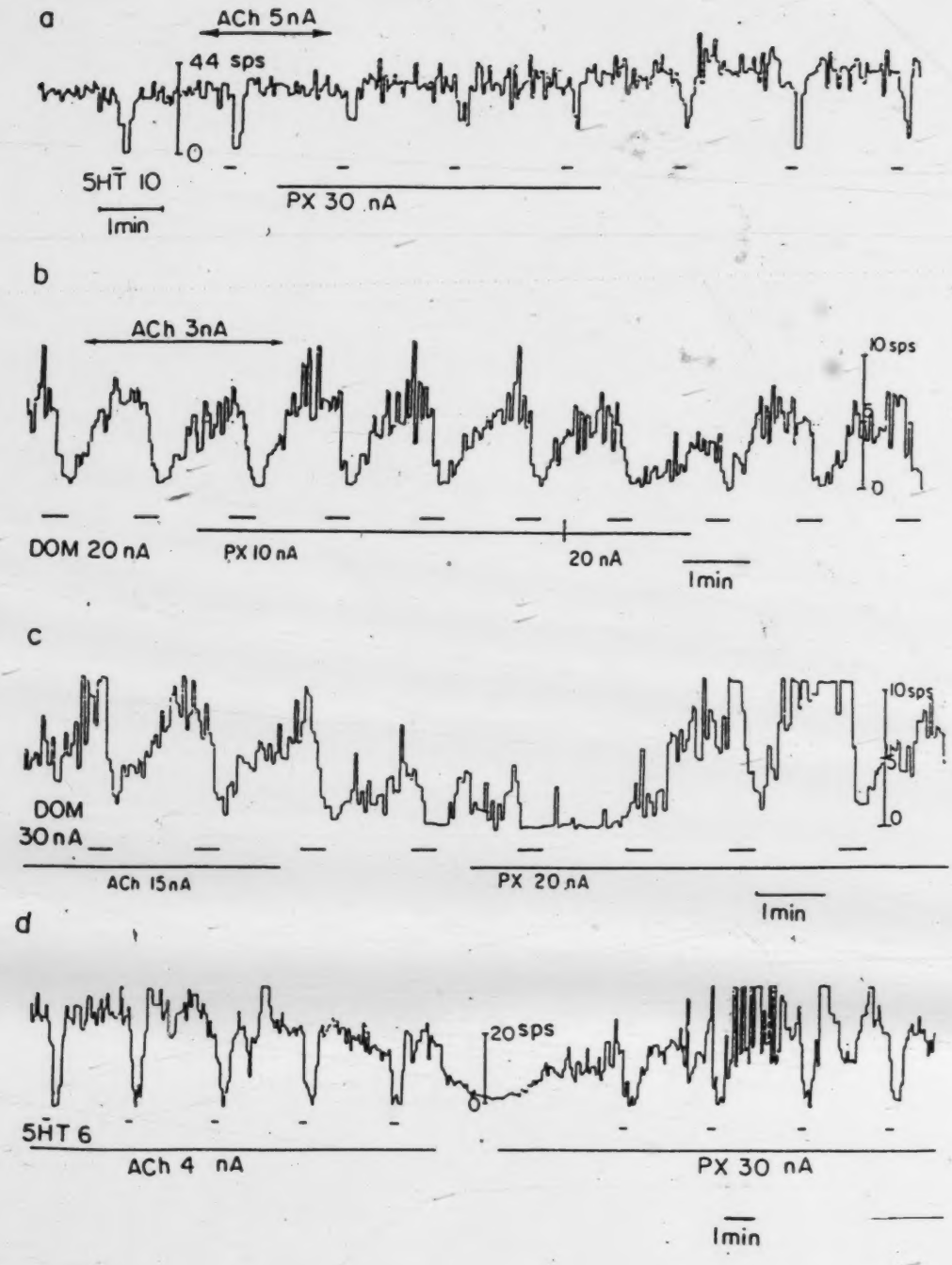
Picrotoxin (the GABA-linked chloride channel antagonist) was tested because of two reports that it was able to prevent the inhibitory effects of 5-HT in the hippocampus (Segal, 1976) and hypothalamus as well as visual cortex (Mayer and Straughan, 1980). The effects of picrotoxin were studied in 4 urethane-anaesthetized animals. Cell firing was maintained by ACh ejection, and a current of picrotoxin which did not further increase the firing rate of the cell was added. 5-HT-induced inhibitions were reduced by about 50% in the presence of picrotoxin (Fig. 11). While the mean (control baseline) firing rate was not altered by the picrotoxin doses used, the pattern of firing became more erratic, with bursts of firing alternating with brief periods of reduced firing. This change to a more irregular

Fig. 11 Effects of Picrotoxin (PX) on 5-HT induced inhibitions of CA_1 neuronal firing maintained by ACh. (a) PX, in a dose which had little effect upon mean single cell firing rate, caused a 50% reduction of 5-HT-induced inhibition. This effect recovered within minutes after PX application ceased. PX did cause the inter-spike interval to appear more irregular, this can be seen in the traces a-d as a more variable base line firing rate between responses to agonist.

(b) DOM-elicited inhibitions were not reduced by PX (n = 4 cells) even though signs of PX ejection were apparent from the firing rate of this cell.

(c) Responses to DOM appear to be of a similar magnitude when cell firing is maintained by ACh ejection as when it is maintained by PX ejection.

(d) In contrast to Fig 9 (a) there is no evidence of reduced effectiveness of the 5-HT-induced inhibition when the ACh maintainance of firing is changed to a PX maintainance of excitation. Note the irregular nature of the firing induced by PX.


a
ACh 5nA
44 sps
0
5HT 10
1min
PX 30 nA
b
ACh 3nA
10sps
5
0
DOM 20 nA
PX 10 nA
20 nA
1min
c
10sps
5
0
DOM
30nA
ACh 15nA
PX 20 nA
1min
d
20sps
0
5HT 6
ACh 4 nA
PX 30 nA
1min

inter-spike interval can be observed in Fig. 11 as a more variable firing rate during picrotoxin application. Since it was possible that the erratic nature of the firing was responsible for the reduced effect of 5-HT (rather than pharmacological antagonism), an additional protocol was followed in 3 of the experiments (Fig. 11a). The cell firing was first maintained by either ACh or picrotoxin and inhibitions were elicited by the application of 5-HT. The continual ejection of the first compound was stopped, and the other compound was then used to maintain the cells at about the previous firing rate. Under these conditions, 5-HT produced a similar degree of inhibition during the ACh-induced firing as it did during picrotoxin-induced firing. This is especially clear for the last two 5-HT-induced inhibitory responses during the ACh- and picrotoxin-induced firing in Fig. 11d, where the same control baseline rates of firing existed. This suggests that the erratic nature of the firing may have been responsible for the reduced effect of 5-HT. Figures 11(b) and (c) show that picrotoxin did not prevent or reduce the inhibitory response to pulses of DOM which produced a large inhibition (but not total suppression) of ACh-maintained CA_1 firing. Similar traces were obtained in 4 animals (4 cells). In Fig. 11(b) where the picrotoxin current was increased to 20 nA, the inhibition produced by DOM appeared to be even longer lasting.

5. The effect of NA, and α and β-adrenoceptor antagonists

As previously reported (Biscoe and Straughan, 1966; Segal and

Bloom, 1974) brief pulses of NA produced a regular reduction in the number of action potentials which normally occurred in response to low currents of ACh or GLU used to maintain firing. The inhibitory effect of Na was not mimicked by ejections of the same or higher currents of Na^+ ions, at the same pH as the NA solution (Fig. 5a). The response of the CA_1 pyramidal cells to NA was always slower in onset and recovery than was the response to 5-HT (see Figs. 12 and 13). As it was possible that 5-HT could have been interacting with α-adrenoceptors (Fenuik, 1984) or even releasing NA to produce its inhibition of firing, the effects of the α-adrenoceptor antagonist phentolamine were examined upon the responses of CA_1 cells to 5-HT and NA (Fig. 12a and b). Even small currents of phentolamine (3 nA) caused large reversible reductions in the baseline control firing rate. When the size of the responses to NA were measured (see Section F.2 of Methods), in the presence of phentolamine the responses were significantly larger; the two inhibitory effects were at least additive (Tables 6 and 7). In three animals phentolamine did not appear to affect the size of responses to 5-HT (Fig. 12b).

D-amphetamine, which presumably acts indirectly by releasing neurotransmitters, inhibited CA_1 firing with a very similar profile to that seen with DOM (Fig. 12c and d). Again the phentolamine-induced inhibition of baseline control firing appeared to be at least additive with that due to D-amphetamine and DOM (Tables 6 and 7), but only significantly so with DOM when responses were measured relative to

Fig. 12 Effect of iontophoretically applied phentolamine (PHEN) on the hippocampal CA_1 neuronal response to various agents.

(a) PHEN caused profound inhibition of CA_1 firing even at low doses. Rather than reduce the size of responses to NA, the PHEN-induced inhibition appeared to be at least additive with that due to NA.

(b) Shows a similar PHEN-induced inhibition of a single CA_1 cell firing record while inhibitory responses to 5-HT were unchanged. Note that inhibition of the control firing rate did not by itself increase the final level of 5-HT-induced inhibition.

(c) Responses to D-amphetamine (D-AMP) were not reduced by PHEN application but were affected in a similar fashion as those to NA (Fig. 10 a).

(d) Responses to DOM were not reduced by PHEN application.

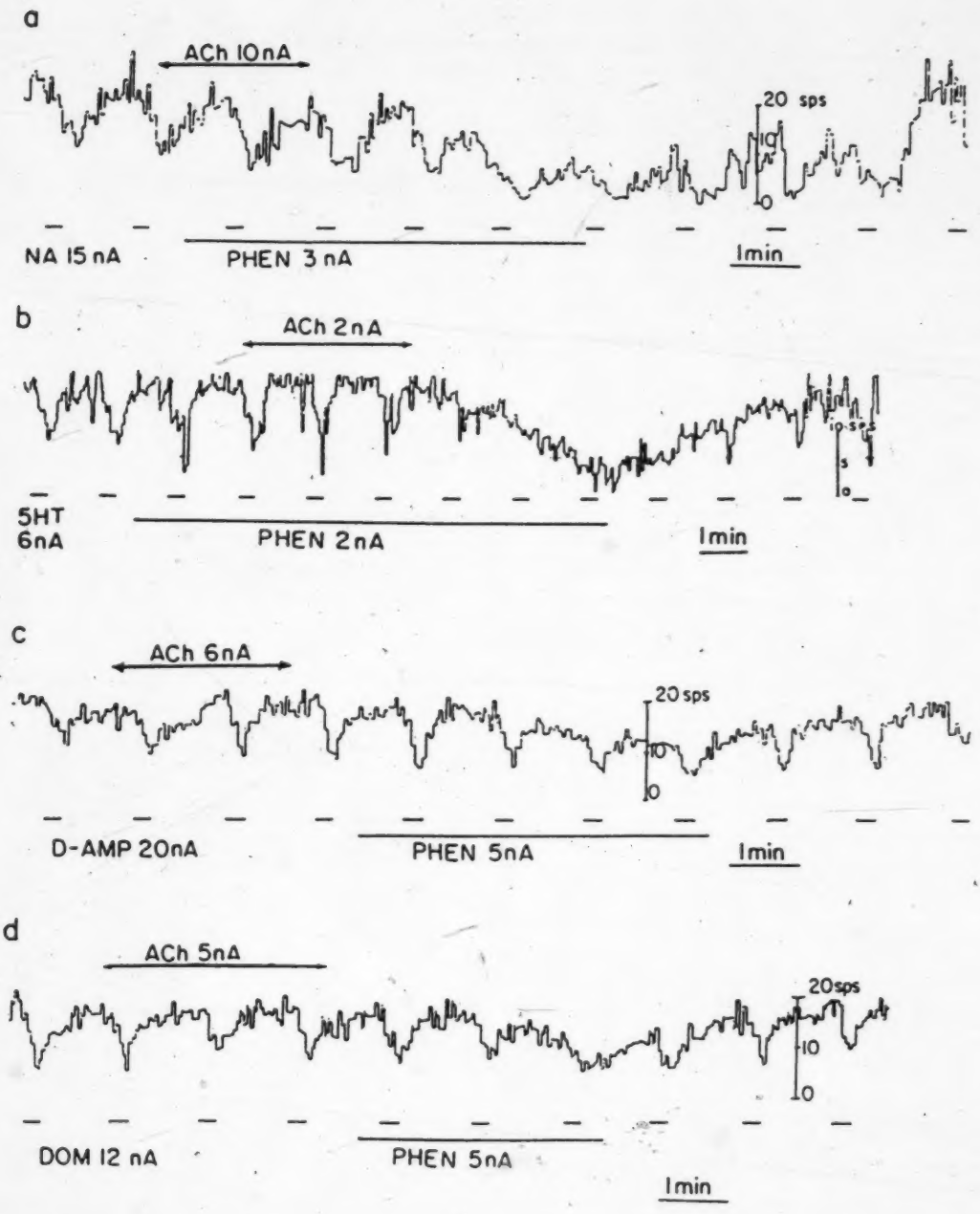

a
ACh 10nA
20 sps
10
0
NA 15 nA
PHEN 3 nA
1min
b
ACh 2nA
10 sps
5
0
5HT
6nA
PHEN 2nA
1min
c
ACh 6nA
20 sps
10
0
D-AMP 20nA
PHEN 5nA
1min
d
ACh 5nA
20sps
10
0
DOM 12 nA
PHEN 5nA
1min

TABLE 6. Adrenergic antagonist effects

Agonists	Antagonists	Trials	Cells	C-T%	SE	t	P
NA	Phentolamine	9	7	-12.0	3.17	3.79	<.01
NA	Sotalol	19	11	+18.72	2.69	6.95	<.001
D-amphet-amine	Phentolamine	19	9	-6.22	1.28	4.85	<.01
D-amphet-amine	Sotalol	12	10	-4.5	2.89	1.56	NS
DOM	Phentolamine	15	6	-2.83	2.46	1.15	NS
DOM	Sotalol	16	6	-6.33	2.59	2.44	NS
DOM	Trifluoperazine	14	7	-5.28	1.50	3.52	<.02

Inhibitions were measured as a percent of the baseline at the start of each inhibitory response. All results were obtained in urethane-anesthetized rats. Only trials where the antagonist dose was sufficient to depress firing are included (with the exception of sotalol data). Inhibition is reduced if the value of C(ontrol)-T(est) is positive. Significance is by "paired t", comparing the inhibition during antagonist with the immediately preceding inhibition ("n" equals the number of cells).

TABLE 7. Adrenergic antagonist effects measured as total inhibition

Agonists	Antagonists	Trials	Cells	C-T%	SE	t	P
NA	Phentolamine	9	7	-16.4	3.06	5.27	<.01
NA	Sotalol	19	11	+16.63	3.74	4.45	<.01
D-amphet-amine	Phentolamine	19	9	-11.44	1.37	8.35	<.001
D-amphet-amine	Sotalol	12	10	-14.3	4.47	3.19	<.02
DOM	Phentolamine	15	6	-9.0	2.79	3.23	<.05
DOM	Sotalol	16	6	-8.0	3.89	2.06	NS
DOM	Trifluoperazine	14	7	-9.14	2.07	4.41	<.01

Inhibitions were measured from the original baseline firing during control inhibitions, and are the sum of reductions in firing due to BOTH antagonist and agonist. This is the appropriate method of estimating antagonism by partial agonists or antagonists (Ariens et al., 1964). Other details are as in Table 6.

Fig. 13 Effect of sotalol on inhibitory responses of CA_1 cells to various agents.

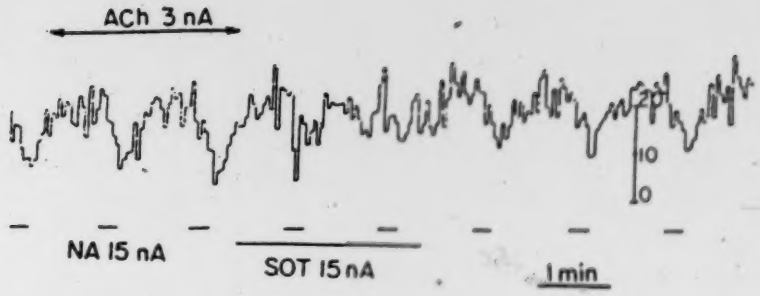
(a) Effect of iontophoretically applied sotalol (SOT) on the hippocampal CA_1 neuronal response to NA. The effect of low currents of SOT on the control firing rate was somewhat reminicent of the effect of picrotoxin (Fig. 9). Overall firing rate increased slightly and became more irregular. The average size of the NA-induced inhibitory response was significantly reduced.

(b) The size of the inhibitory response to 5-HT was not noticeably affected by SOT (n = 3 cells) even though the control firing rate appeared to be more noticeably increased by SOT compared with Fig. 10 (a).

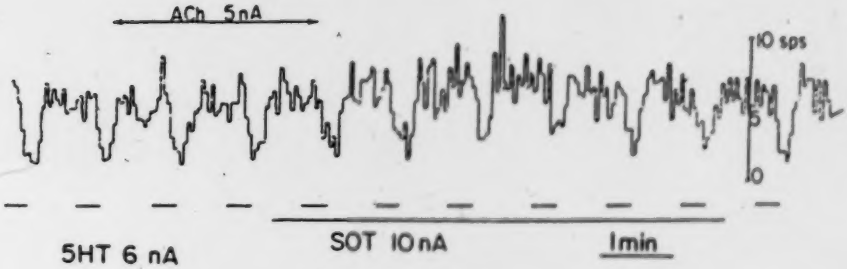
(c) Shows the effect of large currents of SOT (those which decrease control firing rate) on a single CA_1 cell. If enough SOT is ejected there is a reduction in the control firing rate. These amounts (and smaller currents) of SOT did not affect the average magnitude of the DOM-elicited inhibition.

(d) Even large currents of SOT (which depressed firing rate) did not reduce the size of the average inhibitory response to D-amphetamine (D-AMP).

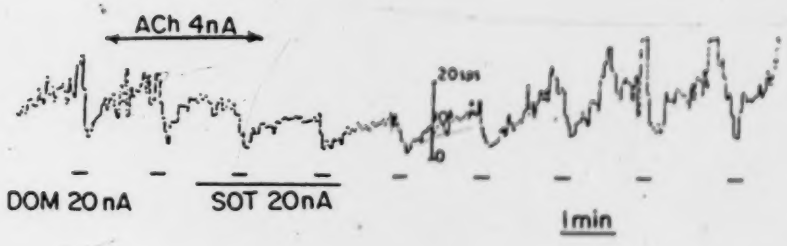
a


ACh 3 nA
20
10
0
NA 15 nA
SOT 15 nA
1 min

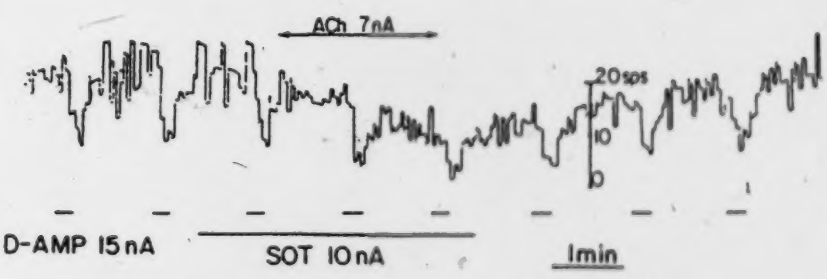
b


ACh 5nA
10 sps
5
0
5HT 6 nA
SOT 10 nA
1 min

c


ACh 4nA
20sps
0
DOM 20 nA
SOT 20nA
1 min

d


ACh 7nA
20sps
10
0
D-AMP 15 nA
SOT 10 nA
1 min

baseline control firing levels (Table 7). Results shown in Fig. 12 and Tables 6 and 7 provide no support for the proposal that responses to NA, 5-HT, D-amphetamine or DOM could be mediated by α-adrenoceptor activation.

It was reported by Segal and Bloom (1974) that the inhibitory response of hippocampal CA_1 pyramidal cells to NA could be prevented by the iontophoretic application of the β-adrenoceptor antagonist sotalol. In Fig. 13(a) it can be seen that some reduction in the size of the response to NA does occur upon application of sotalol. This can also be seen when the results of all experiments are averaged (Tables 6 and 7); by either method of measurement, the response to NA is always 15-20% smaller. Sotalol did not reduce the baseline control firing rate (at the currents used), but did appear to cause a small increase in the overall rate of firing and change the firing pattern in a manner reminiscent to that seen with picrotoxin. In 3 control animals sotalol did not reduce the 5-HT-mediated response, even though the baseline control firing rate was slightly elevated by the sotalol. Low currents of sotalol which slightly increased baseline control firing rate, and large currents which decreased it, failed to reduce the inhibition caused by D-amphetamine or DOM (Figs. 13c and d and Tables 6 and 7). The responses to D-amphetamine and DOM were actually a little larger in the presence of sotalol, but this difference did not reach significance except with the interaction between sotalol and D-amphetamine measured and expressed relative to

the original control baseline (Table 7). Results shown in Fig. 13 and Tables 6 and 7 provide no evidence for the proposal that responses to DOM or D-amphetamine could be mediated by β-adrenoceptor activation.

As the response to DOM may have been mediated by DA receptors, the neuroleptics trifluoperazine (n=7) (Fig. 14) and chlorpromazine (n=5) were examined for an antagonist effect on responses of CA_1 cells to DOM. It is clear from Fig. 14 that trifluoperazine depressed the baseline firing rate of the CA_1 cells, but the responses to DOM were not decreased. By both methods of measurement, the inhibition due to trifluoperazine and DOM appeared to be at least additive (Tables 6 and 7). Chlorpromazine was tested on one CA_1 cell whose firing rate was maintained by ACh, and on 4 cells whose firing was maintained by GLU in order to control for any anti-muscarinic effect of chlorpromazine. Although the GLU-maintained baseline firing rate was irregular, rendering measurement of the responses difficult, no indication of antagonism of the responses to DOM was observed, despite local anaesthetic effects at high currents of chlorpromazine.

The effect of intravenous drugs on the blood pressure and the firing rate of single CA_1 pyramidal cells is shown in Fig. 15. The effects of a miscellaneous collection of drugs upon the firing rate of locus coeruleus (LC) cells and hippocampal CA_1 cells is discussed in Appendix II. As the time course of firing changes seemed to follow quite well the change in blood pressure, very few experiments were done using the intravenous route. Figure 15(a) illustrates the

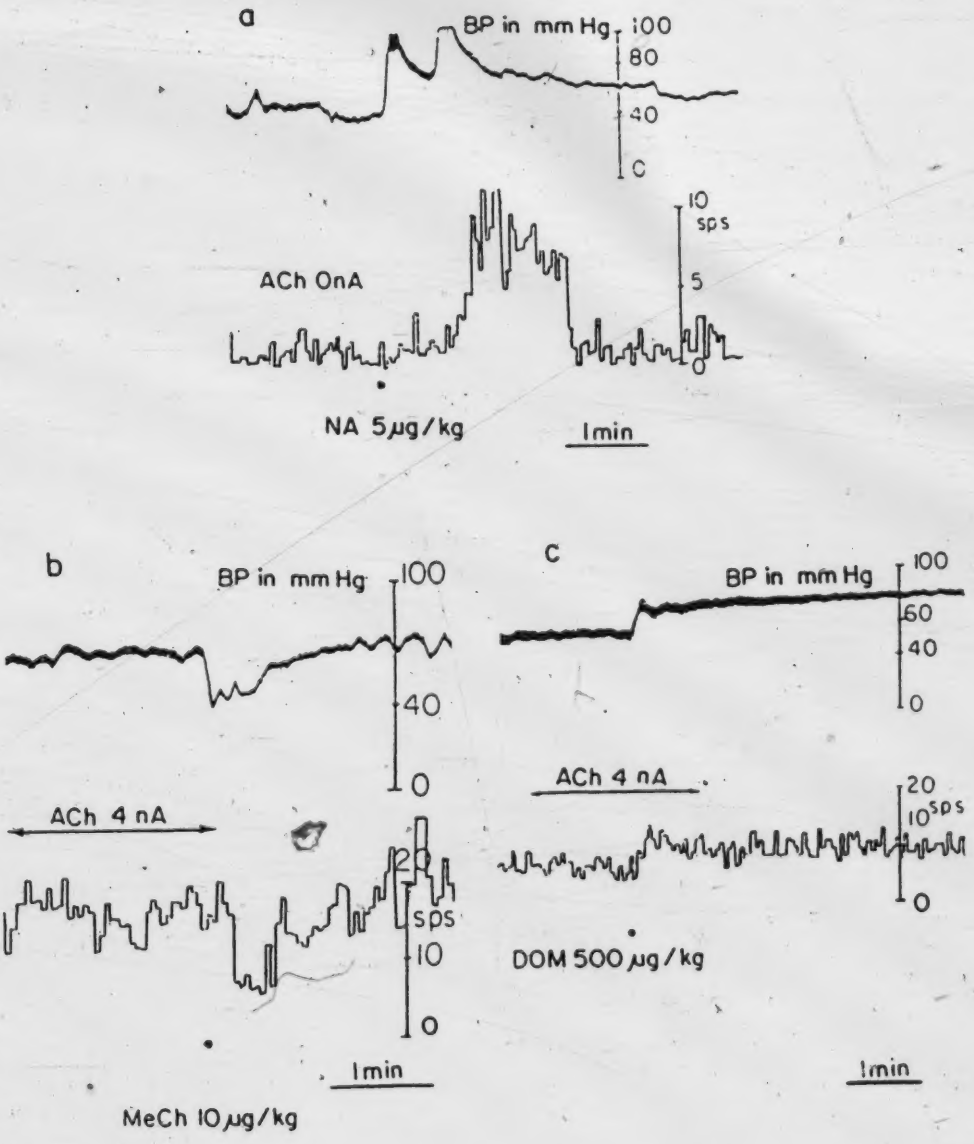
Fig. 14 Illustrates the effect of iontophoretically applied trifluoperazine (TFP) on the hippocampal CA_1 single cell response to DOM. TFP reduced the control firing rate, but did not reduce the average magnitude of the DOM-elicited inhibition. Instead the TFP-induced inhibition was on average at least additive with that due to DOM.

Fig. 15 The effects of blood pressure alteration on hippocampal CA_1 cell firing.

(a) Blood pressure recording from an anaesthetized male Sprague Dawley rat (upper traces) and firing rate of a single hippocampal CA_1 cell (lower traces). An intravenous injection of noradrenaline (NA) (which should not enter the brain) caused a large increase in blood pressure; this was followed shortly afterwards by a large increase in cell firing rate which lasted as long as the blood pressure increase.

(b) An intravenous injection of methacholine (MeCh) (which also should not enter the brain) produced a brief fall in blood pressure which was accompanied by a fall in CA_1 cell firing of a similar duration.

(c) Intravenous injection of DOM caused a rise in blood pressure and an increase in CA_1 single cell firing rate; the excitation of the hippocampal cell showed a similar time course to the blood pressure changes.


a
BP in mm Hg
100
80
40
0
10
sps
5
0
ACh OnA
NA 5µg/kg
1min
b
BP in mm Hg
100
40
0
ACh 4 nA
20
sps
10
0
1min
MeCh 10µg/kg
c
BP in mm Hg
100
60
40
0
ACh 4 nA
20
10 sps
0
DOM 500µg/kg
1min

response of CA_1 pyramidal cells to i.v. NA. In 5 animals 1-5 µg/kg NA (which should not cross the blood-brain barrier) produced a large but short-lasting rise in the blood pressure. In Fig. 15 it can be seen that the blood pressure rise has two phases; this is because the NA was washed in slowly and was not given as a bolus. The usual response of the CA_1 cell was a delayed increase in firing rate, with no change in the action potential size (which may indicate cell movement relative to the electrode). The time course of this response followed the duration of the blood pressure change. Conversely the injection of 10 µg/kg methacholine produced a short reduction in the blood pressure (Fig. 15b); this was followed by a brief cessation of the firing of the cell (n=2 cells). At this point it is worth noting that the firing of LC cells demonstrated the inverse correlation with blood pressure alteration (Persson and Svensson, 1981; this thesis, Appendix II). Drugs which lowered the blood pressure excited LC cells and drugs which raised the blood pressure inhibited LC spontaneous activity. These changes in LC firing could, in turn, inversely influence CA_1 cell firing.

In 5 animals injection of DOM (0.1-0.5 mg/kg) produced a rise in blood pressure which was accompanied by a rise in CA_1 cell firing rate (Fig. 15c).

B. Recordings from dorsal raphé cells

The effect of DOM on the DR, putative serotonergic neurones of the rat brain, was assessed as the ability to inhibit the spontaneous

firing rate of these cells is a property which is shared by proposed 5-HT autoreceptor agonists such as LSD and 5-MeoDMT (de Montigny and Aghajanian, 1977). An indirectly acting agonist such as D-amphetamine might be expected to inhibit the firing of these cells by the release of a neurotransmitter; a likely candidate to mediate this action would be 5-HT (Wang and Aghajanian, 1978).

The natural phenethylamine hallucinogen mescaline has no inhibitory agonist action upon DR cells (Haigler and Aghajanian, 1973). Thus, the effect of a drug on DR units may be an index of whether it is LSD-, amphetamine-, or mescaline-like.

1. Iontophoretically applied compounds

In total, 42 5-HT responsive cells were presumed to be serotonergic by the criteria described in Methods, Section D. Twenty-four of these recording sites were stained successfully, and histological examination revealed that all of them were located within the DR nucleus. Eight cells were located in the dorsal part of the nucleus, 10 in the central and 6 in the ventral region. By reference to Table 8 which is a summary of the data, it can be seen that 5-HT produced a profound average inhibitory response (78%) in 35 cells (Fig. 16a) at very low ejection currents (average 7.6 nA). The inhibition produced by 5-HT was usually associated with an increase in the amplitude (peak to peak) of the extracellularly recorded action

TABLE 8. The effects of iontophoretically applied compounds on dorsal raphé units.

	DOM	D-amphetamine	Mescaline	5-HT
% inhibition [a]	20±3(n=17)	52±7(n=16)	4±2(n=7)	78±3(n=35)
Ejection current [b]	16±1 nA	17±1 nA	18±1 nA	7.6±0.4 nA
Fraction inhibited	14/17	15/16	3/7 [d]	35/35
No response	3/17	1/16	4/7 [e]	0/35
IT_{50} [c]	-	675±17(n=7)	-	86±7(n=33)

[a] Mean ± S.E.M. inhibition of baseline firing rate.

[b] Mean ± S.E.M.

[c] Current (I) x time to 50% inhibition ± S.E.M.

[d] Two of these cells showed small increases in spike size.

[e] Local anesthetic effects observed.

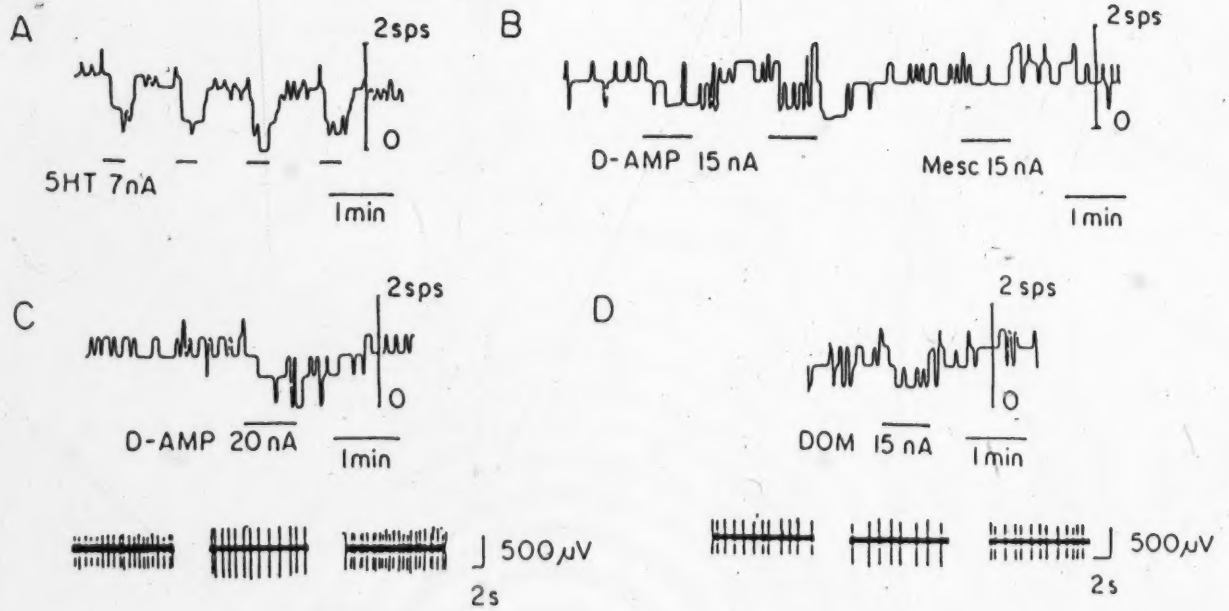
Fig. 16 Effects of phenethylamines on dorsal raphé cells. Records of the firing of four neurones and their responses to the iontophoretic application of various compounds.

(a) Typical inhibitory response to 5-HT.

(b) Inhibitory responses to D-amphetamine and the lack of response to mescaline.

(c) Response to D-amphetamine with photographs showing action potential size before, during, and after drug administration. The photographic records have been re-touched to remove the halo effect of the oscilloscope beam.

(d) Weak inhibitory effect of DOM also accompanied by an increase in action potential size.


A
2sps
0
5HT 7nA
1min
B
2sps
0
D-AMP 15 nA
Mesc 15 nA
1min
C
2sps
0
D-AMP 20 nA
1min
500μV
2s
D
2 sps
0
DOM 15 nA
1min
500μV
2s

potential (indicative of hyperpolarization, Humphrey, 1979). Higher currents of application resulted in total suppression of DR neurone firing. As greater than 50% inhibition could be achieved, a current x time to 50% inhibition value was calculated which for 5-HT was 86 ± 7 coulombs. Mescaline produced only a negligible average inhibition in the absence of local anaesthetic effects; larger currents caused a long-lasting depression of spike size (indicative of local anaesthetic effects (Curtis, 1968)). The firing of several cells was reduced by a maximum of 10%; these were in the ventral DR. This result is in general agreement with that of Haigler and Aghajanian (1973) except that in their study there was no difference between the responses of cells in different regions of the nucleus. DOM application produced an average maximal inhibition of 20% (n=17 cells). Larger amounts of DOM often caused loss of cell recording. In 75% of cases inhibitory responses to DOM were accompanied by increases in spike amplitude (see Fig. 16d). There was no difference in the inhibitory effectiveness of DOM, whether the cells were centrally or ventrally located within the DR (central cell average inhibition 18%, ventral 20%).

D-Amphetamine produced an average inhibitory response of 52% for 16 cells (Table 8; Fig. 16b and c) although four of these cells were maximally inhibited. The inhibitory responses to D-amphetamine application occurred consistently, and had an $I.T._{50}$ value of 675 ± 17 coulombs. All the cells responding to D-amphetamine with inhibition

did so with an increased spike size. Although the drug was only tested on dorsally and centrally located DR units, no difference between the responsiveness of these cells was observed. None of the compounds tested on DR firing produced an increase in cell firing rate.

2. The effect of intravenous compounds

DOM by the i.v. route (05-0.5 mg/kg in physiological saline), injected slowly, inhibited the firing of 5 dorsal and centrally located cells, however the period of inhibition coincided exactly with large blood pressure increases (Fig. 19). One of these cells which was inhibited did not show recovery of cell firing (Fig. 17a). D-Amphetamine (0.1-0.25 mg/kg in saline) produced similar effects with three cells (Fig. 20) (in one case firing did not recover) and no alteration of firing rate with 3 other cells where the blood pressure increase was not as large (Fig. 17a). These cells were also dorsally and centrally located. LSD (25 µg/kg in saline) inhibited one centrally and one ventrally located DR unit with little associated change in blood pressure (see Fig. 18). Figure 20 illustrates the effect of D-amphetamine applied by the i.v., and direct microiontophoretic routes to the same cell. On this cell D-amphetamine (25 nA) caused about a 40% inhibition of control rate, but Na^+ ions at the same pH applied at the same current for almost twice the duration of the D-amphetamine application had no effect. D-Amphetamine by the i.v. route (0.2 mg/kg) produced a similar response to that due to iontophoretic D-amphetamine, but the duration of the inhibition

Fig. 17 The effects of intravenous D-amphetamine (D-AMP) and DOM on dorsal raphé (DR) cell firing and blood pressure. Blood pressure records (upper trace) and histograms of single unit firing of a DR neurone (lower traces).

(a) In this experiment an intravenous injection of D-AMP produced a large transient rise in blood pressure but did not alter the firing rate of this cell (n = 3 cells). An injection of the same weight (free base) of DOM caused a larger transient increase in blood pressure, accompanied by a cessation of DR firing. In this case cell firing did not recover (the cell firing was integrated over a shorter time interval).

(b) In a similar experiment, the injection of the same volume of 0.9% NaCl produced a smaller rise in blood pressure as did the injection of D-AMP. The cell stopped firing after the D-AMP (n = 3 cells) but again in this experiment firing did not recover.

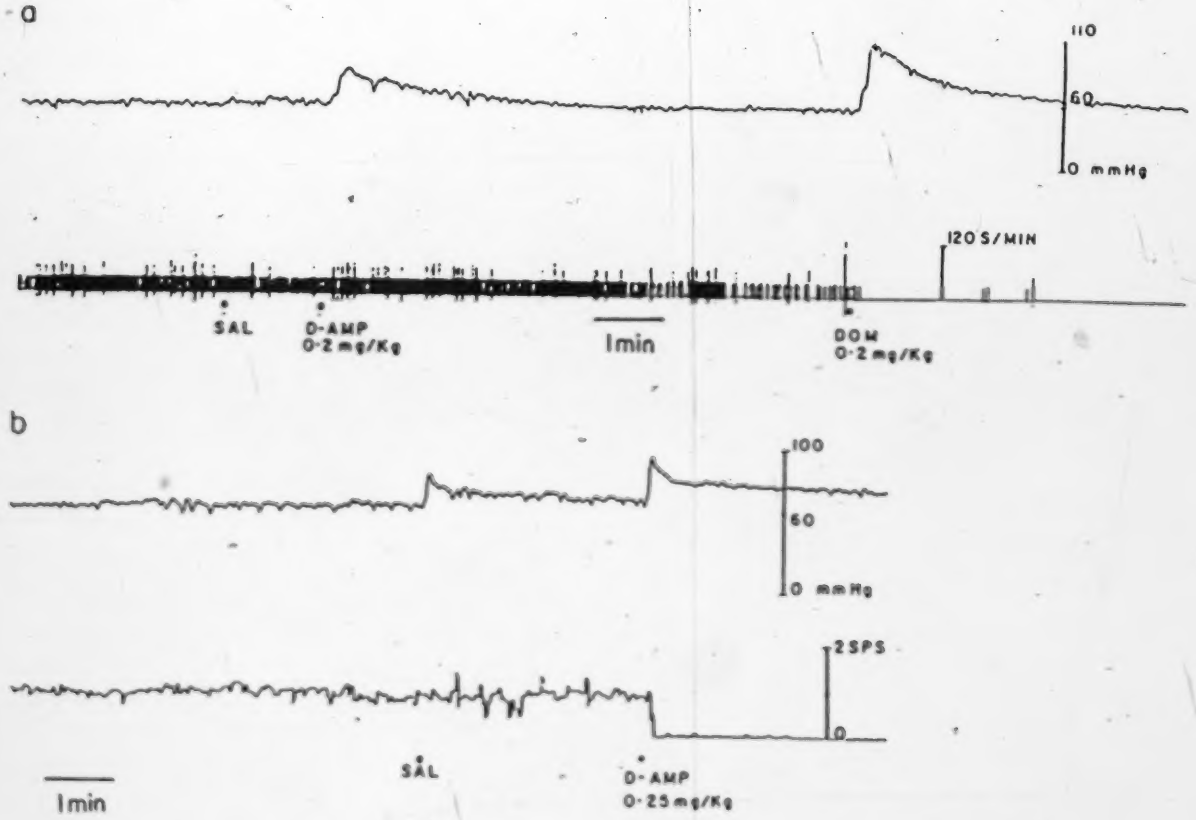
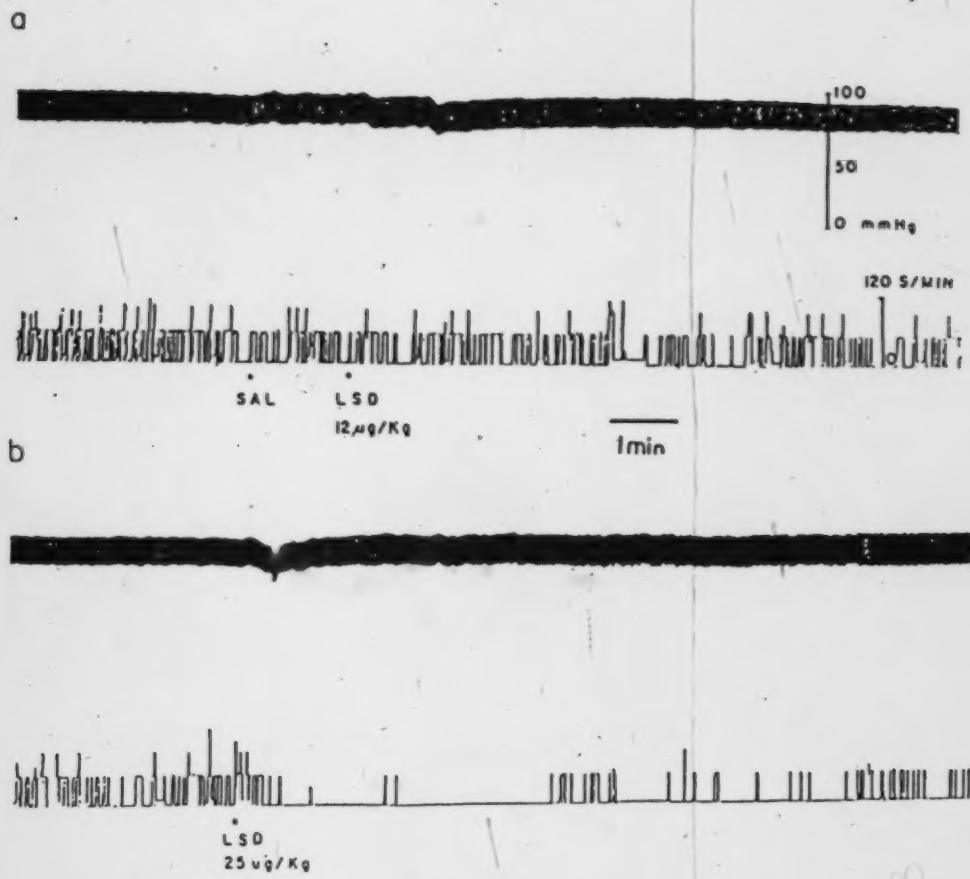

a
110
60
0 mmHg
120 S/MIN
SAL
D-AMP
0·2 mg/Kg
1min
DOM
0·2 mg/Kg
b
100
60
0 mmHg
2SPS
0
SAL
D-AMP
0·25 mg/Kg
1min

Fig. 18 The effect of intravenous LSD on dorsal raphé (DR) firing rate and blood pressure. Blood pressure records (upper trace) and histograms of single unit firing of a DR neurone (lower traces).

(a) An intravenous injection of 12 µg/kg of LSD produced only a very small decrease in the blood pressure, and only a slight inhibition of DR firing rate.

(b) 25 µg/kg of LSD however produced an inhibition of DR firing rate which lasted for about 5 min. There was only a small reduction in blood pressure (n = 2 cells). Traces (a) and (b) are from the same cell.


a
100
50
0 mmHg
120 S/MIN
SAL
LSD
12µg/Kg
1min
b
LSD
25 µg/Kg

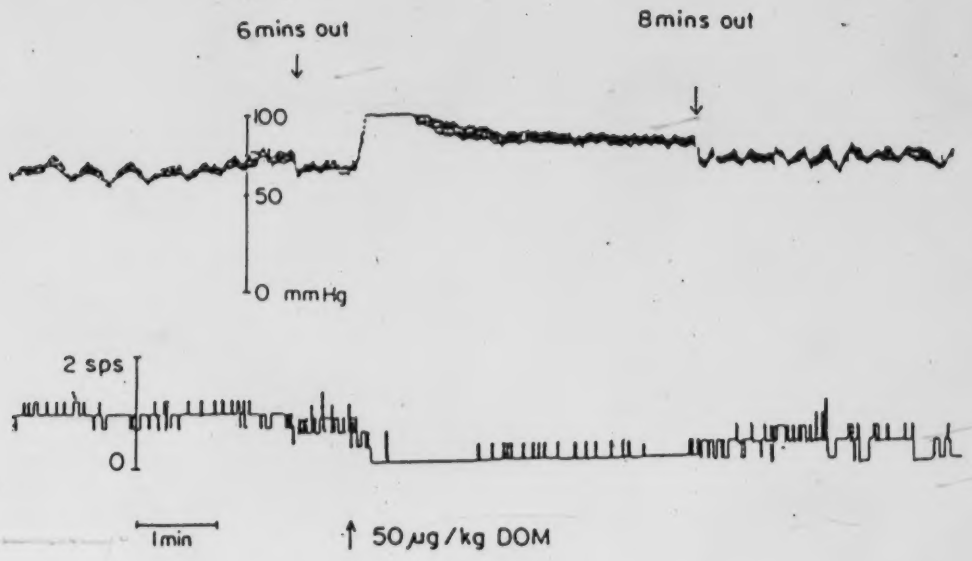
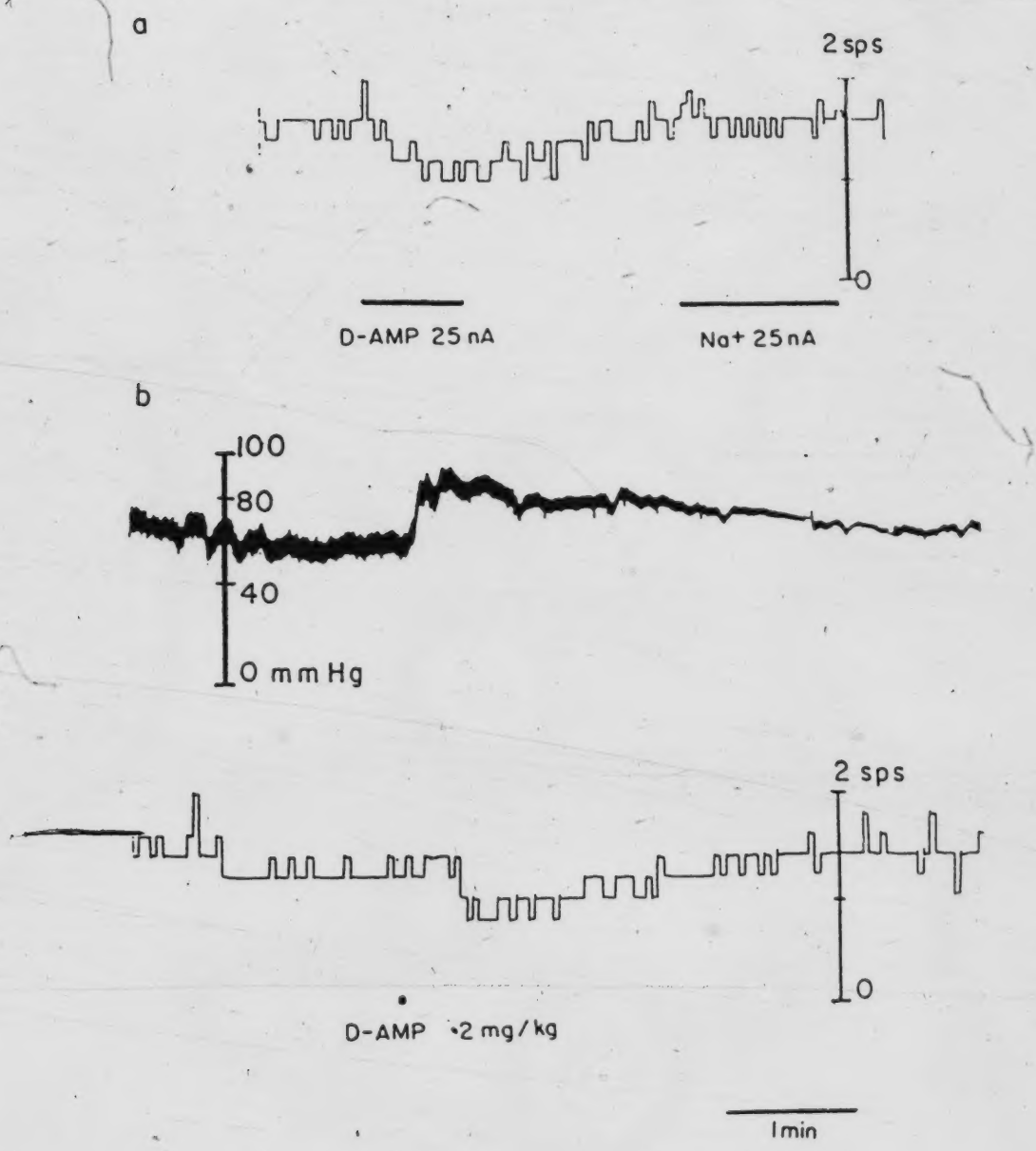


Fig. 19 The effect of intravenous DOM on (DR) firing rate and blood pressure. Blood pressure record (upper trace) and histogram of the firing of a single unit in the DR nucleus (lower trace). An intravenous injection of 50 µg/kg DOM HCl produced a large increase in blood pressure which remained elevated for about 10 min. The effect on blood pressure was accompanied by a reduction in firing of this cell. The time course of the two effects was similar (n = 5 cells).

Fig. 20 Effects of iontophoretically applied and intravenous D-amphetamine (D-AMP) on a dorsal raphé (DR) neurone.

(a) Firing rate histogram of a single DR cell. D-AMP produced a slowing of the firing of this cell but the same current value of Na^+ ions ejected for even longer than the D-AMP, had no effect.

(b) In the same cell, an intravenous injection of D-AMP produced a transient rise in blood pressure which was accompanied by a similar inhibitory response as seen in (a). The time course of the blood pressure and firing rate changes was similar.


a
2 sps
0
D-AMP 25 nA
Na+ 25 nA
b
100
80
40
0 mm Hg
2 sps
0
D-AMP 2 mg/kg
1 min

closely followed the duration of the associated blood pressure increase.

C. Recordings from facial motor neurones

It has been suggested that 5-HT_2 type receptors may mediate the excitatory responses to 5-HT of central neurones (Davis and Roberts, 1985). For this reason, the effect of directly applied DOM to cells which usually increase their firing rate in response to 5-HT, and where the effects of 5-HT can be selectively antagonized, was examined. Such a site was discovered by McCall and Aghajanian (1979a, 1979b, 1980a, 1980b) who characterized the response of brainstem FMN's to 5-HT and NA. In order to observe the excitation of FMN's produced by 5-HT or NA when recording extracellularly, this group showed that a small excitatory input to the cells provided by subthreshold synaptic activation or antidromic stimulation is required. Alternatively, an ejection of GLU, several times lower than the threshold current of GLU required to cause cell firing, can be used.

1. The facilitation of FMN firing by 5-HT, NA, DA and DOM

Recordings from a total of 69 cells (from 69 animals) were made, 49 of these recording sites were stained successfully and histological examination showed that all of them were located within the facial motor nucleus. As reported by McCall and Aghajanian (1979a), FMN's when provided with small amounts of 5-HT or NA show greatly facilitated excitatory effects of iontophoretically applied GLU (Figs. 21, 22 and 27b). 5-HT applied with currents in the range 10-25

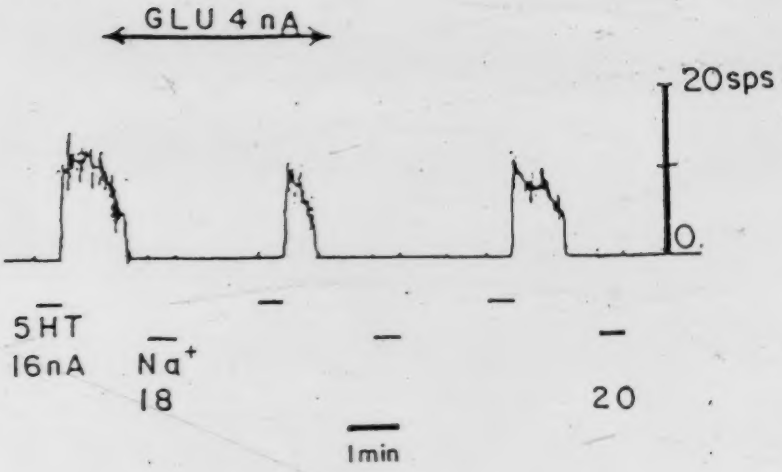
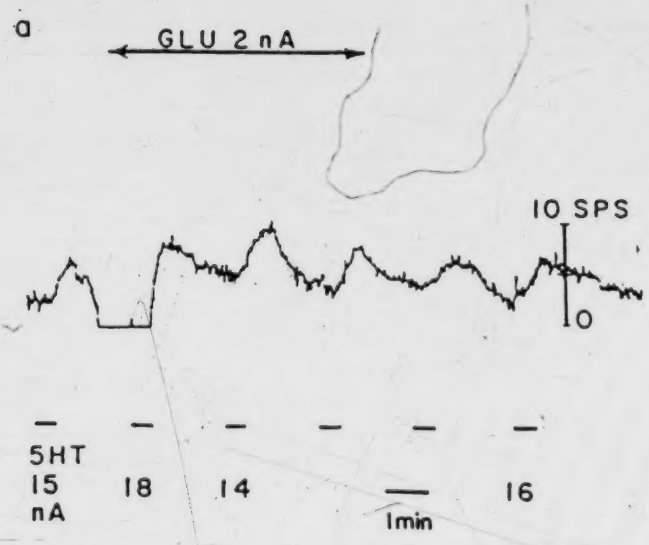


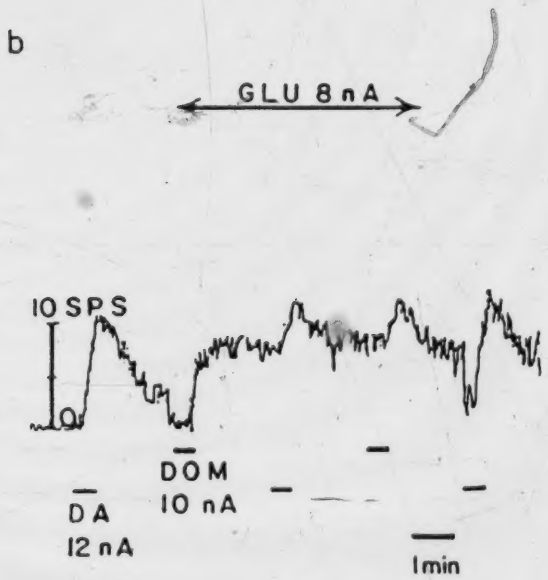
Fig. 21 Firing rate histogram from a single facial motor neurone (FMN) and response to 5-HT. As in following traces, glutamate (GLU) was ejected for the duration of the trace in amounts that were smaller than threshold currents required for the initiation of firing (about 20 nA). 5-HT application produced a large increase in firing rate which developed slowly; the cell had usually started to fire before the end of the 30 second 5-HT application. Similar or larger currents of Na^+ ions at the same pH as the 5-HT did not cause excitation.

Fig. 22 Effect of 5-HT, DA and DOM on a spontaneously firing facial motor neurone (FMN).

(a) Illustrates the relatively unusual occurrance of a FMN which fired continuously in response to a small current of glutamate (GLU). When the cell was silent, 5-HT activated it; the effect of 5-HT on the already firing cell can be seen to be a slowly developing increase in the rate of its discharge.

(b) Similar sized current pulses of DA produced an activation of this FMN as did a small pulse of DOM; these excitations were similar in shape and duration to the ones caused by 5-HT. During the final response to DA there appears to be a small pre-excitation inhibition. This phemonenon was seen only occasionally with the application of most agonists studied.


a
GLU 2 nA
10 SPS
0
5HT
15
nA
18
14
16
1min


b
GLU 8 nA
10 SPS
0
DOM
10 nA
DA
12 nA
1min

nA caused all cells to which it was applied (51 out of 51) to increase their firing rate. The excitatory response often reached its maximum near the end of the period of 5-HT application, and frequently outlasted it by a factor of at least two (Fig. 23). If the GLU current was turned off, the responses to 5-HT did not occur. Routine Na^+ current ejection from the current balance barrel (at the same pH as the 5-HT) did not cause excitatory responses (Fig. 21). NA was applied to 13 cells and in each case it produced a similar response to that of 5-HT application (Fig. 26c and 27b). The action of DA had not previously been tested on FMN units; it was found that 27 of 28 cells responded with excitation to pulses of DA (Fig. 22). Some difficulty was encountered when attempts to alternate responses to NA and DA were made. There was a tendency to lose quickly the response to one or the other putative transmitter. This problem did not arise when responses to 5-HT and DA, or 5-HT and NA were alternated. DOM application with the same duration (30 sec) pulses also facilitated the firing of FMN units on 35 of 37 cells (Fig. 22b and 24b). The duration of DOM-induced firing was similar to that caused by NA, DA and 5-HT; also similar maximal frequencies of firing were attained (10-15 SPS). The constancy of the excitatory response of FMN's to DOM and DA application (when they were alternated) was not well maintained; this was reminiscent of the problems which occurred with responses to NA and DA; however after considerable effort two successful experiments were performed.

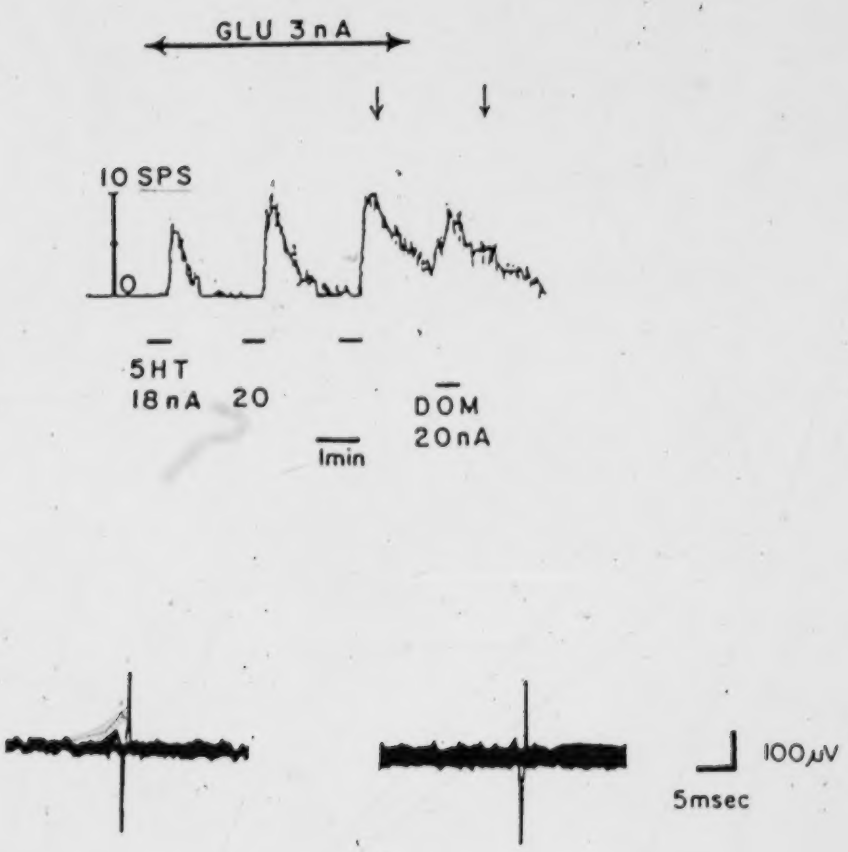


Fig. 23 Illustrates excitatory responses of a facial motor neurone (FMN) to the application of 5-HT and DOM. Below the histogram are photographs of single unit action potentials. The downward pointing arrow on the left in the upper trace indicates the time at which the left photograph was taken; the one on the right corresponds to the time at which the night photograph was taken. Note that 5-HT and DOH elicit action potentials of an almost identical size (peak to peak).

2. The effect of 5-HT "D" antagonists

Small currents of methysergide (average 8 nA applied usually for 5 min or less) completely prevented the excitatory response to 5-HT without altering the size or the shape of the action potential (Fig. 24a and Tables 9 and 10). For all 4 cells out of 7 where responses to NA were alternately elicited with those to 5-HT (Fig. 27b), the responses to 5-HT were antagonized but those to NA were completely unaffected (Tables 9 and 10). Similarly, responses to DOM were antagonized by methysergide on all 5 cells tested. On 3 of these cells the specificity of the antagonism of DOM was assessed by comparing the antagonist effect on responses to NA; the latter responses were unaffected (Fig. 26c). Records from two cells where DOM and 5-HT pulses were alternated showed that methysergide antagonized the effect of both compounds; in addition the recovery of both responses had the same time course (Figs. 26b and 27a). On 4 cells methysergide antagonized responses to 5-HT but on 3 out of these 4 cells the control agonist response to DA was also reduced by a similar amount. Methysergide was found to prevent selectively excitation induced by 5-HT when compared with responses to NA. However when the effects of methysergide were compared on responses to 5-HT and DA, then usually antagonism by methysergide showed no such selectivity for responses to 5-HT (Tables 9 and 10). The one experiment where methysergide selectively prevented responses to 5-HT and left responses to DA relatively unaffected is shown in Fig. 26(a).

TABLE 9. The proportion of facial motor neurone excitatory responses that were prevented by various antagonists.

	ANTAGONISTS			
Agonist	Methysergide	Chlorpromazine	Ketanserin	Cinanserin
5-HT	11/11	0/8	3/3	1/1
NA	0/7	-	1/1	-
DA	3/4	10/10	1/1	1/1
DOM	5/5	0/5*	-	-

Table showing the proportion of facial motor neurone excitatory responses that were prevented by various antagonists. The denominator represents the total number of cells to which the antagonist was applied. The effects of these antagonists were reversible, and an index of their specificity was obtained by noting their action on the response to a control agonist.

*Any depression in the size of the response to DOM was either accompanied by a depression in spike size (not evident during 5-HT or DA elicited responses), or was essentially irreversible in the sense that a reduction in the response was still present after full recovery of the response to DA.

TABLE 10. Facial motor neurone firing during antagonism and recovery as a percentage of control.

Antagonist	Agonist	n(cells)	During Antagonism	Recovery	Mean Antagonist Current nA
	5-HT	11	23 ± 7	87 ± 3	8
	NA	7	101 ± 3	100 ± 5	8
Methysergide	DA	4	39 ± 21	74 ± 20	6
	DOM	5	18 ± 7	89 ± 13	8
	5-HT	8	98 ± 1	100 ± 1	8
Chlorpromazine	DA	10	25 ± 7	96 ± 3	6
	DOM	5*	60 ± 19	38 ± 11	7
	5-HT	3	12	100	10
Ketanserin	DA	1	0	100	8
	NA	1	50	100	16
Cinanserin	DA	1	20	95	5
	5-HT	1	25	100	5

Values are expressed as mean ± S.E.M.

*Effects of chlorpromazine on responses to DOM were measured at a time either just prior to spike depression of the 5-HT control response, or at the time when the control response to DA was antagonized. Only one cell was studied per animal.

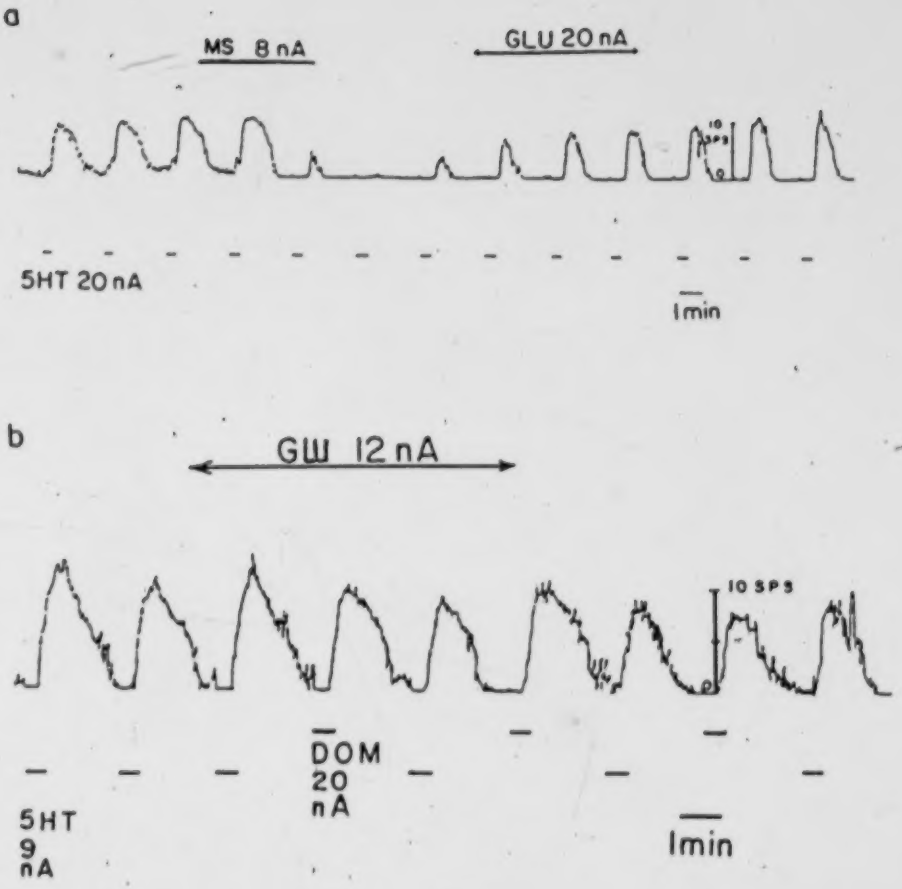


Fig. 24 Antagonism of the excitatory response to 5-HT of a facial motor neurone (FMN) by methysergide (MS).

(a) Illustrates the firing of a single FMN in response to pulses of 5-HT. A small current of MS, a "D" 5-HT receptor antagonist, was enough to reversibly antagonize the 5-HT elicited excitation.

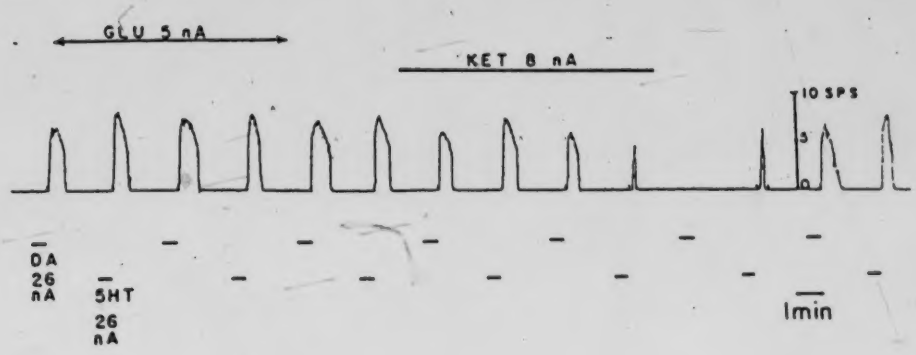
(b) Long-lasting 5-HT-elicited firing which commenced before the termination of the 5-HT current pulse. DOM caused a similar activation of this cell.

Fig. 25 Antagonism of facial motor neurone (FMN) excitatory responses to DA and 5-HT by ketanserin (KET).

(a) Shows 5-HT-and DA-elicited excitatory responses of a FMN which are regular in amplitude and duration. Application of the 5-HT_2 antagonist KET reversibly decreased the magnitude of the excitatory responses to these substances.

(b) KET reduced responses to DA and 5-HT with a longer time course (the KET was applied for longer). The response to DA appeared to take a little longer to recover than did the response to 5-HT. The two traces in Fig. 25 (b) are continuous.

a


GLU 5 nA
KET 8 nA
10 SPS
5
0
DA
26
nA
5HT
26
nA
1min

b

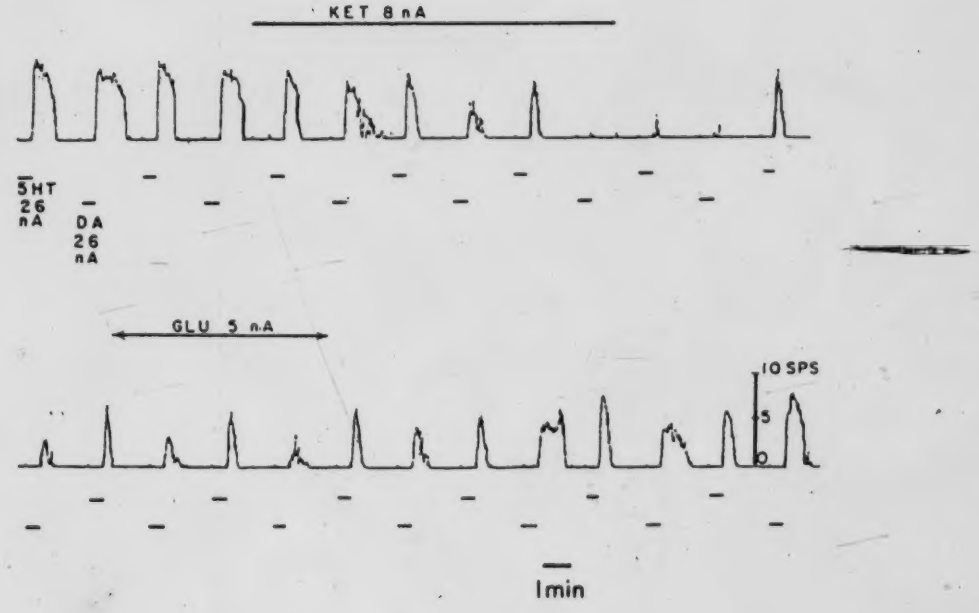

KET 8 nA
5HT
26
nA
DA
26
nA
GLU 5 nA
10 SPS
5
0
1min

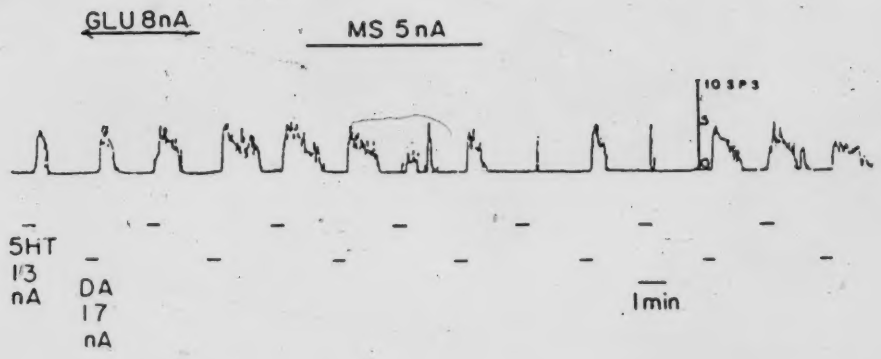
Fig. 26 The effect of methysergide (MS) on responses of a facial motor neurone (FMN) to 5-HT, NA, DA and DOM.

(a) This trace illustrates responses from the one cell out of four in which MS application produced a more profound antagonism of the response to 5-HT than it did of the response to DA. The more usual result was that responses to DA and 5-HT were reduced in parallel.

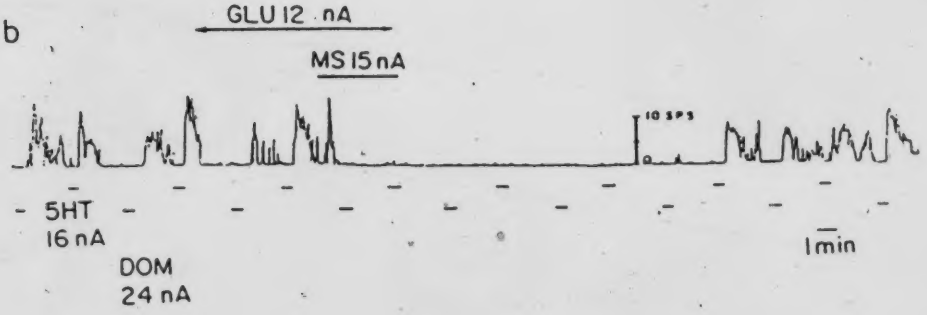
(b) MS antagonized responses to DOM and 5-HT in parallel, responses recovered together.

(c) MS reversibly reduced responses to DOM at currents that had no effect upon the control response to NA.

a


GLU 8nA
MS 5nA
10 SPS
5HT
13
nA
DA
17
nA
1min

b


GLU 12 nA
MS 15nA
10 SPS
5HT
16 nA
DOM
24 nA
1min

c

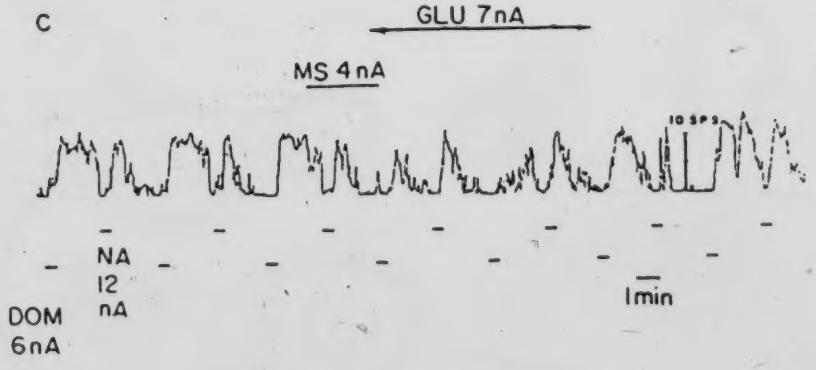
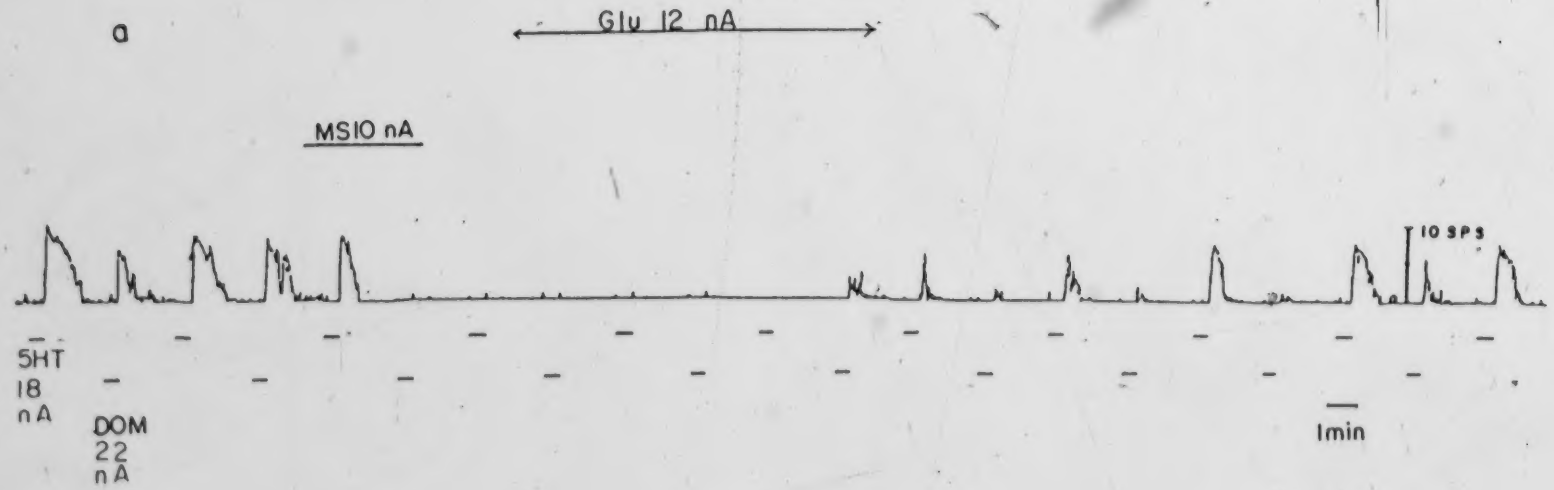

GLU 7nA
MS 4nA
10 SPS
NA
12
nA
DOM
6nA
1min

Fig. 27 Effect of methysergide (MS) on responses of a facial motor neurone (FMN) to 5-HT, DOM and NA: an assessment of the selectivity of MS.

(a) Excitatory responses of a FMN to DOM and 5-HT, MS caused reduction of both responses in parallel. They start to recover together, but in this experiment recovery of the responses to 5-HT appeared sooner than did responses to DOM.

(b) MS antagonized completely the excitatory response of this FMN to 5-HT whilst no effect upon responses to NA was observed. In this cell about the time the response to 5-HT started to recover it can be seen that the responses to NA had declined a little. This may have been due to a change in the excitability of the cell with time.


a
Glu 12 nA
MS10 nA
10 SPS
5HT
18
nA
DOM
22
nA
1min

b
Glu 20 nA
MS 10nA
10 SPS
NA
14
nA
5HT 20
nA
1min

The 5-HT_2-selective antagonist ketanserin reversibly antagonized the response to 5-HT with no effect upon the action potential size or shape (n=3 cells). However, ketanserin was also effective in reducing the size of responses to NA and DA (Fig. 25a and b and Tables 9 and 10). Results depicted in Figure 25(b) suggest that the response to DA may take a little longer to recover from antagonism by ketanserin than does the response to 5-HT.

One experiment was performed using the "D" antagonist cinanserin. As previously reported (McCall and Aghajanian, 1980a) this compound antagonized responses to 5-HT, but also reduced the responses to DA which were alternated with 5-HT (Tables 9 and 10).

3. The effect of chlorpromazine on responses to 5-HT, DA and DOM

The neuroleptic chlorpromazine antagonized the response to DA of 10 cells (Tables 9 and 10; Fig. 28a) without any noticeable effect upon the action potential size or shape. On all 5 of these cells where the control agonist 5-HT (alternated with the DA pulses) was tested, chlorpromazine had no effect on 5-HT-elicited firing or action potentials. However, the effects of chlorpromazine on responses to DOM appeared to be quite complex. Where DA was alternated with DOM, the response to DA was completely antagonized by chlorpromazine, but a reduction in the response to DOM was also apparent (Fig. 28b) (n=2 cells). The responses to DOM showed poor recovery, which occurred some time after full recovery of the responses to DA (Fig. 30). Where responses to DOM were alternated with responses to 5-HT (n=3 cells), it was clear that currents of chlorpromazine (which had no effect

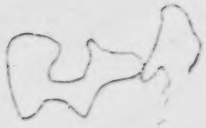


Fig. 28 The effect of chlorpromazine (CPZ) on responses to DA, 5-HT, and DOM.

(a) Shows 5-HT- and DA-elicited excitatory responses of a facial motor neurone (FMN) which are regular in amplitude and duration. Application of the anti-psychotic CPZ reversibly antagonized the excitatory response to DA while the response to 5-HT was unaffected.

(b) On this FMN CPZ completely antagonized the response to DA but a reduction in the response to DOM was also apparent.

(c) Low currents of CPZ (which had no effect upon the response to 5-HT) also did not affect the response to DOM. However just after the break in the bar, where the CPZ current was increased from 5 to 8 nA, a local anaesthetic effect on the 5-HT-elicited action potential was noted. At this point a similar effect on the DOM-elicited spike was also seen; this may have contributed to the reduction in DOM-elicited firing by CPZ. The local anaesthetic effect on the 5-HT-elicited spike wore off quicker than did the effect on the DOM-elicited spike.

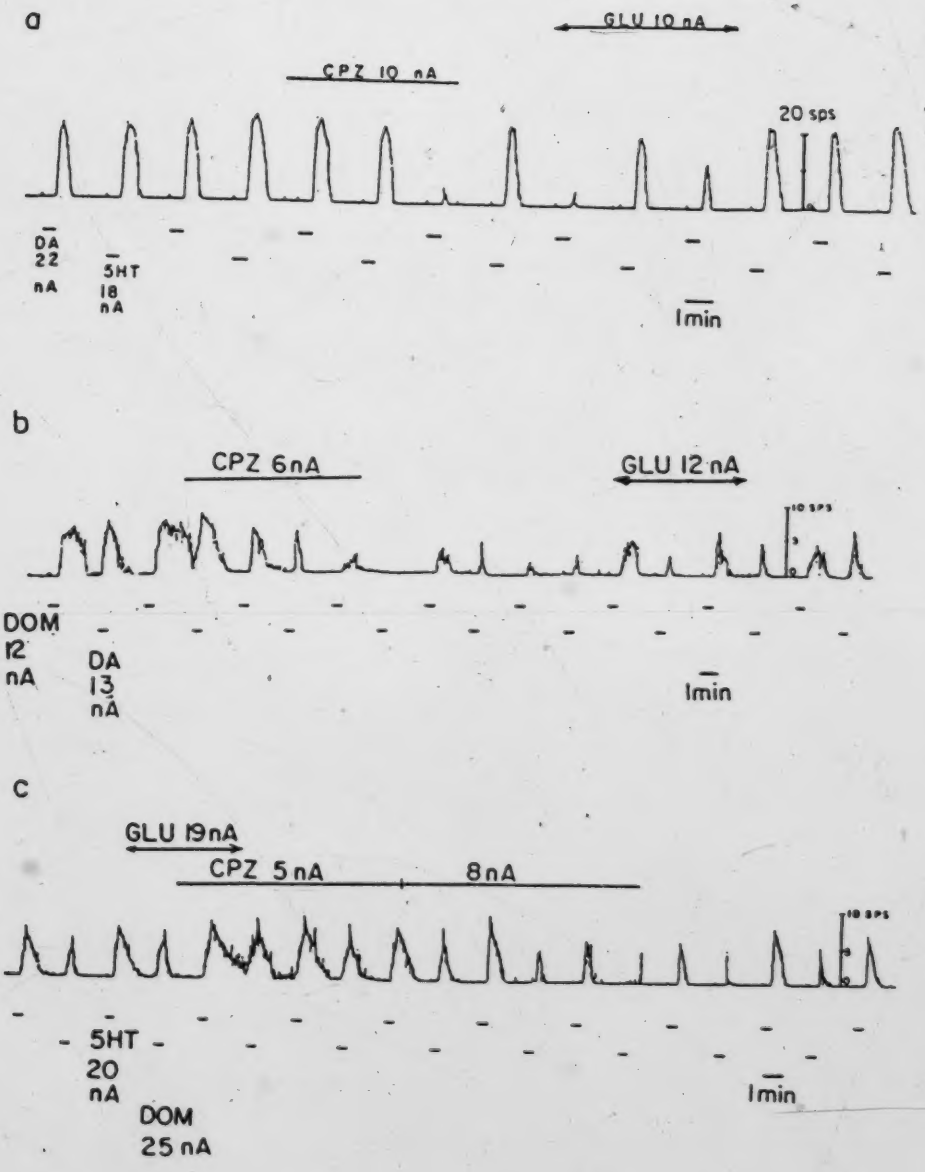
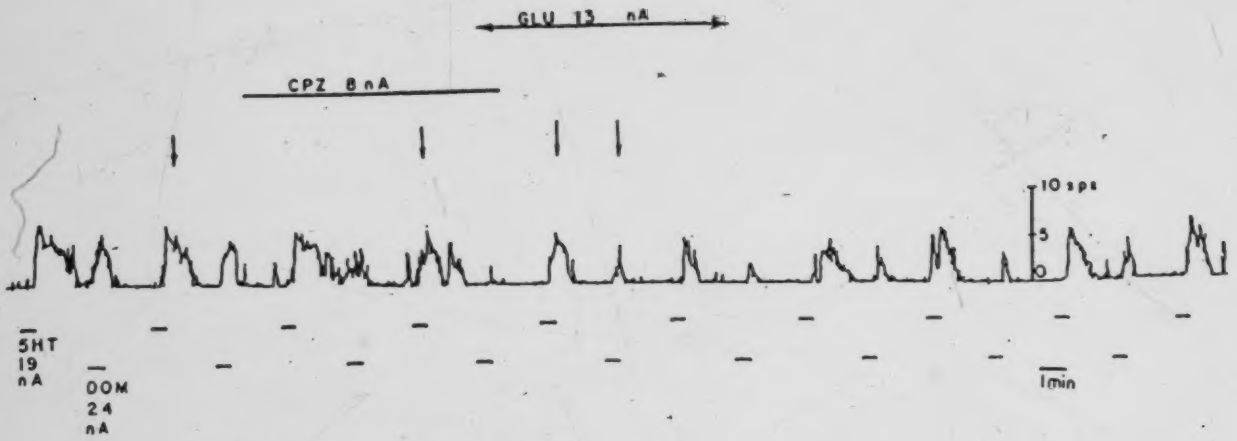

a
GLU 10 nA
CPZ 10 nA
20 sps
DA
22
nA
5HT
18
nA
1min
b
CPZ 6nA
GLU 12 nA
DOM
12
nA
DA
13
nA
1min
c
GLU 19nA
CPZ 5 nA
8nA
5HT
20
nA
DOM
25 nA
1min

Fig. 29 The effect of chlorpromazine (CPZ) on the amplitude of action potentials elicited by 5-HT and DOH. Excitatory responses of a single facial motor neurone (FMN) to DOH and 5-HT. As in Fig. 28 (c) low currents of CPZ had no effect upon the excitatory responses. However near the end of the CPZ application local anaesthetic effects were noted. The four downward pointing vertical arrows from left to right indicate the times at which the respective photographs of the action potentials were taken. The first photograph shows two control spikes elicited by 5-HT (DOH-elicited spikes were identical). The second photographshows a depression of action potential size of the 5-HT-elicited spike. This had recovered by the third arrow to a supra-control level. The photograph corresponding to the fourth arrow demonstrates that the local anaesthetic effect of CPZ on DOH-elicited spikes was considerably longer lasting than were its effects upon the 5-HT-elicited action potentials.


GLU 13 nA
CPZ 8 nA
10 sps
5
0
5HT
19
nA
DOM
24
nA
1min

100μV
5msec

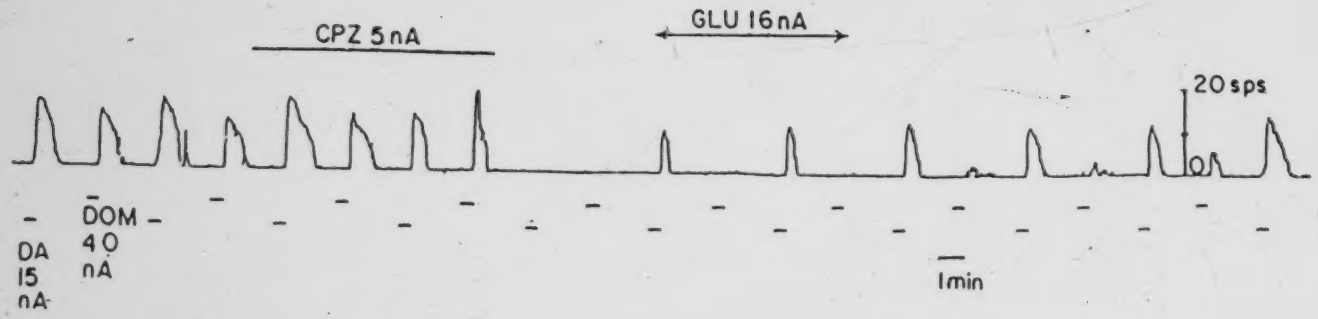


Fig. 30 Effects of chlorpromazine (CPZ) on responses of a facial otor neurone (FMN) to DA and DOM: time course of recovery. CPZ antagonized completely the response to DA which started to recover after about 2 min post CPZ. The response to DOM was also reduced but it did not start to recover until about 14 min after the termination of the CPZ current. When the response to DA had almost reached its full control level the response to DOM had recovered by only about 40%.

upon the response to 5-HT) did not affect the response to DOM. Higher currents of chlorpromazine produced a local anaesthetic effect on the 5-HT-elicited action potential. A similar effect upon the DOM-elicited spike was also seen (Fig. 28c and 29); this may have contributed to the chlorpromazine-caused reduction in the size of the response to DOM when alternated with DA. It is noteworthy that the local anaesthetic effect on the 5-HT-elicited action potential wore off quicker than did the effect on the DOM-elicited spike. Where enough chlorpromazine had been given to produce a reduction in the spike amplitude elicited by 5-HT, then recovery of the response to DOM was always poor (Figs. 28c, 29 and Table 10).

CHAPTER IV

DISCUSSION

A. Experiments on hippocampal CA_1 cells

1. The effects of 5-HT and putative antagonists

Iontophoretically applied 5-HT inhibited the spontaneous, ACh or GLU-maintained firing of hippocampal CA_1 cells as reported previously in several studies (Biscoe and Straughan, 1966; Segal, 1975, 1976, 1980; Blier and de Montigny, 1983). Investigations in which 5-HT has produced inhibition of central neurones, and attempts to prevent this with antagonists, have been reviewed in the Introduction (G.1). Data described in Results (A.3), support and extend the generalization that 5-HT "D" receptor antagonists, which are effective at peripheral 5-HT receptors in smooth muscle (Gyermek, 1961), do not produce any observable reduction in the inhibitory effect of 5-HT upon extracellularly recorded cell firing in the central nervous system. A recent review (Fozard, 1984) points out that there also appear to be no selective receptor antagonists for the neuronal inhibitory or smooth muscle relaxing effects of 5-HT in the periphery. Hippocampal CA_1 cells may be added to the areas listed by Haigler and Aghajanian (1974) where all 5-HT antagonists tested to date have been ineffective at preventing 5-HT-induced inhibition: ventral LGN, optic tectum, amygdala, DR nucleus, and reticular formation. All these areas receive innervation by 5-HT-containing neurones (Azmitia, 1978), but until effective and selective receptor antagonists are found some doubt will remain about the role of this innervation.

The type of responses observed in this study highlight some of

the problems of measuring antagonism of inhibition when the putative antagonists themselves appear to have significant intrinsic activity (or suppress firing by an unrelated mechanism). The ways in which responses were measured have been described in Methods, section F.2(i). Several methods of expressing antagonism could have been employed:

(a) The absolute amplitude of the response to the inhibitor could be measured, regardless of any change in the baseline firing and compared as a percentage of the response in the absence of the "antagonist". This is a conventional method, which is in error if there is any reduction in baseline firing caused by the "antagonist" as this could occlude the agonist-induced inhibition.

(b) A second method makes limited adjustments for baseline changes. All inhibitions are expressed first as a percentage of the firing rate at the beginning of the individual response (the amount of inhibition possible), and this is compared with the response (measured in the same way) obtained in the presence of the antagonist". This method will also give spurious results if the "antagonist" causes a pronounced depression of baseline firing (See Fig. 31).

(c) A third method takes into account any depression of baseline firing. Inhibitions are measured as the total produced by both agonist and antagonist, expressed as a percentage of the original

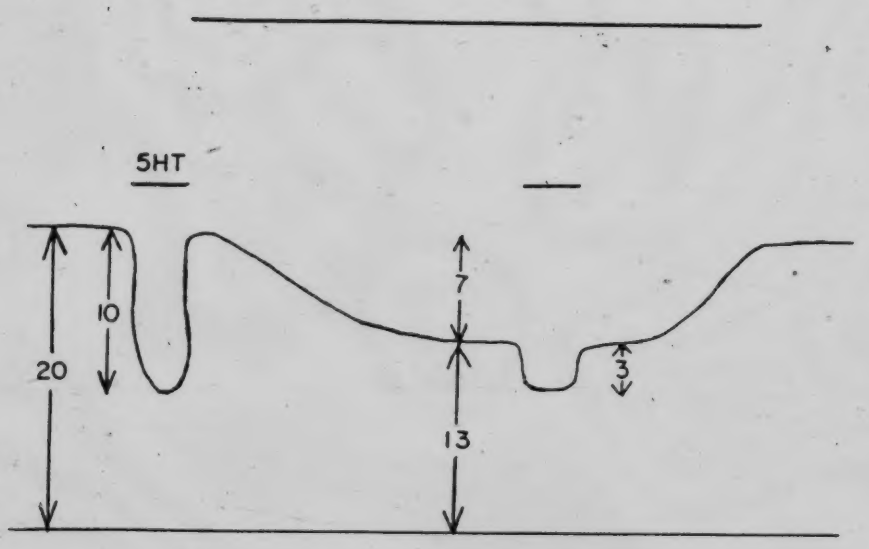


Fig. 31 Illustration of a hypothetical response to 5-HT of a cell's firing rate, and one in the presence of a putative antagonist which reduces baseline control firing rate. If 5-HT inhibits from a baseline firing rate of 20 spikes per sec down to 10/sec, then the response is 50%. However if a putative antagonist, by any mechanism, causes a reduction in rate of 7 spikes per sec to 13 per sec, with no change in the final level of inhibition then the response goes to 3/13 or 23%. If baseline inhibition in the absence of an antagonist does not increase the total inhibition from baseline, then the apparent reduction in the inhibition (50 to 23%) would not represent antagonism of the agonist response.

baseline (i.e. as a percentage of the total inhibition possible).

Method "c" is the most appropriate for the measurement of the responses observed in this section. If one assumes that the reduction of the baseline firing induced by the antagonist is independent of a serotonergic mechanism, then this reduction is cancelled out by the method. An important consideration is that baseline depression itself does not increase the total level of inhibition caused by the agonist, a condition which was usually observed with miscellaneous drugs and spontaneous reductions in baseline firing of CA_1 cells. If one assumes that the "antagonists" are acting on 5-HT receptors to produce the baseline inhibition, then the drugs must be partial agonists/antagonists or full agonists as they cannot be pure antagonists. If this is the case, then total inhibition (that due to agonist plus antagonist method "c", Ariens et al., 1964) should be measured. Ideally, the proof of partial antagonism is that the maximal response of the agonist plus the maximal response of the partial antagonist is the maximal response of the partial antagonist. Unfortunately it was not possible to carry out this ideal test, as both agonist and "antagonist" could produce a maximal (100%) inhibition; therefore it was not possible to ascertain the maximal effect of either. The measurement of membrane potential changes of the cell is clearly required to measure the maximal effect. Several points agree with the result of measurements made, and confirm that antagonism of 5-HT did not occur:

(i) Suppression of baseline control firing by agents other than

putative antagonists, i.e. DOM or 5-HT, does not increase the final level of inhibition caused by 5-HT. If this was not the case, and the putative antagonists did not increase the final level of 5-HT mediated inhibition (as they do, see below), then this could have been considered as evidence of antagonism.

(ii) In many cases inhibition caused by 5-HT was actually greater in the presence of the antagonist, significantly so in the case of methysergide applied in the halothane-anaesthetized rat (measured by method "c").

(iii) In the situation where conventional measurement (type b) would bias data towards antagonism (Fig. 31), no significant antagonism was found (Table 4); this could only be the case if greater inhibition by 5-HT during putative antagonist application occurred.

(iv) Another way to observe the expression of possible antagonism is to measure time to half-maximal inhibition (Simmonds, 1974). This could not be accurately done with the type of integrator, the time-base used, and the relatively rapid onset of the response. From most figures it can be seen that inhibitory responses of CA_1 cells to 5-HT were very fast in onset; this was not obviously changed by the putative antagonists and therefore suggests no antagonism. While data obtained in this study may suggest that the putative antagonists are in fact full agonists, neither this nor a partial agonist action is provable in the

absence of any clear antagonist.

It is difficult to determine why our results are so consistently different from those of Segal (1976). This was true in several different test conditions: using halothane for anaesthesia, and ACh to maintain firing, to duplicate exactly experimental conditions of Segal, or when urethane was used for anaesthesia and firing was maintained with ACh or GLU. Segal (1976), appears to have used measurement technique "a" (above) but he reported no measurements or statistical analysis other than the number of cells affected. Two of Segal's records (Figs. 3B,C in Segal, 1976) show clearly that 5-HT, which caused maximal inhibition in the control, still caused maximal inhibition throughout cyproheptadine and Brom-LSD applications (the former also severely suppressed cell firing). No selectivity of antagonism was demonstrated, in the sense that the antagonists were not tested against other inhibitory neurotransmitters, for example GABA or NA (nor were other types of antagonists used).

All the "D" antagonists could completely suppress firing so the currents of antagonists used were, of necessity, lower than those which would completely occlude the 5-HT-induced inhibition. Nevertheless, it is likely that sufficient drug was ejected to show possible antagonism of the response to 5-HT since the doses used (a) always caused some suppression of firing, and (b) did show some indication of antagonism of the after-excitation which followed 5-HT applications. An appropriate test for selectivity of this action

would examine the effects of 5-HT "D" antagonists upon after-excitations caused by NA. As it is, the only information available which has a bearing on this point is that not all drugs which inhibit baseline (control) firing rate prevent after-excitation.

The finding that ketanserin had no observable antagonistic properties when tested on inhibitory responses to 5-HT was expected given the results of Kohler (1984) who found that ketanserin bound only sparsely in the hippocampus in a manner which suggested that there are no 5-HT_2 type receptors on hippocampal CA_1 cells. It is likely that inhibitory responses to 5-HT, where they have been studied, are not mediated by 5-HT_2 receptors, as these responses were not antagonized, but specifically potentiated by ketanserin (Lakoski and Aghajanian, 1985).

LSD has effects on whole brain 5-HT turnover which suggest a 5-HT receptor agonist profile (see Introduction D); it appeared to behave as an agonist on hippocampal CA_1 firing (although this cannot yet be proven until selective antagonists are available). In this regard it is interesting to note that classical "D" type 5-HT antagonists generally fail to produce any significant increase in the turnover of 5-HT in the brain (D'Amico et al., 1976; Jacoby et al., 1978), but have different effects on 5-HT levels in different brain areas, possibly depending on the relative presence of different 5-HT receptor types (i.e. 5-HT_1 or 5-HT_2) (Ugresic and Draskoci, 1983).

As phenoxybenzamine will irreversibly alkylate the smooth muscle

"D" 5-HT receptor (Gaddum and Picarelli, 1957), it seemed reasonable to test phenoxybenzamine on hippocampal CA_1 cells. It is critical that phenoxybenzamine is at the correct pH for the active form of the drug to be ejected (Graham, 1957); however, phenoxybenzamine came out of solution at pH's higher than 3.6. Thus when the drug is ejected into neutral CSF it is not clear whether the active form occurs. Nonetheless, Baraban and Aghajanian (1980) have reported that phenoxybenzamine ejected from an identical solution to the one used in this study had effects upon DR cells which were reversible. Bevan et al. (1977) have also reported that phenoxybenzamine was a selective, quickly reversible antagonist of α_1-agonist-mediated excitation of cortical cells. Inhibitory responses of cortical cells to α_1-agonists were not antagonized. The irreversible phase of antagonism by phenoxybenzamine probably takes some time to occur, as early effects of phenoxybenzamine are reversible (Nickerson, 1962). To allow for this, currents of phenoxybenzamine were left on for up to 30 min; however, no decrement in the response to 5-HT could be observed. The simplest conclusion to be drawn from this result is that the response is not mediated by receptors similar to the smooth muscle "D" type.

The new tropine ester of cocaine "M" antagonist (see Introduction, B.1) MDL 72222 (Fozard, 1984) also failed to prevent the inhibitory response to 5-HT of CA_1 pyramidal cells. This is not surprising in the light of findings by Richardson et al. (1985); this group showed that the more potent indoletropanyl esters of cocaine, though they act like MDL 72222 in the periphery, have no appreciable

affinity for rat uterine "D", or cerebral cortical 5-HT_1 or 5-HT_2 binding sites.

2. Experiments with picrotoxin

Results of the experiments with picrotoxin confirm that a reduction in the size of the inhibitory response to 5-HT can be obtained with application of this substance to cells whose firing is maintained by ACh. However, as the pattern of firing of the cells was altered, this effect may not be selective for just 5-HT and GABA. If the effect of picrotoxin is to be compared on inhibitory responses to two transmitters, in order to evaluate the specificity of any interaction with the response to one of the transmitters, then a candidate which produces a similar response to 5-HT with respect to its quick time course of action should be chosen. This follows from the proposed mechanism of action of picrotoxin in causing excitation of CA_1 cells. Picrotoxin is thought to block chloride channels linked to GABA receptors, thus blocking the effect of recurrent inhibition due to GABA release. Hablitz (1984) has shown in the hippocampal slice preparation that picrotoxin has little or no effect on the passive properties of the cell membrane; however, the effect of excitatory inputs are greatly potentiated. This was attributed to a [temporal] summation of recurrent excitatory inputs in the situation where the GABAergic I.P.S.P. amplitudes are reduced, this could explain the erratic firing after picrotoxin; i.e.: the result of a summation of excitatory and inhibitory inputs, of both ephaptic and synaptic nature (Taylor et al., 1984). Consequently, the longer the

time course of a response to an inhibitory agent, the more opportunity exists for interruption of that response by the effects of an undamped excitatory input. The inhibitory effect of glycine has a quick time course, but it could not be used as a control agonist as it has only weak effects on hippocampal CA_1 cells (Ben-Ari et al., 1981a). Another candidate would have been GABA itself; however, its effects would have been antagonized, thus underlining the lack of specificity of the picrotoxin effect on 5-HT-induced inhibition.

When the CA_1 pyramidal cell firing was maintained by picrotoxin ejection alone, the inhibition due to 5-HT was unaffected by the presence of the picrotoxin. This result suggests that 5-HT is probably not acting either indirectly, by releasing GABA, nor directly on the same chloride channels affected by GABA and picrotoxin. The fact that picrotoxin exerted considerable excitation by itself is evidence that GABA-induced inhibition was largely blocked without the action of 5-HT being affected.

ACh and picrotoxin may cause excitation of CA_1 pyramidal cells by different mechanisms. ACh, acting on muscarinic receptors can facilitate the firing of pyramidal cells by two different mechanisms: a slow postsynaptic depression of K^+ conductance (Dodd et al., 1981; Ben-Ari et al., 1981b); and a fast onset presynaptic suppression of inhibitory control (Krnjevic et al., 1981), possibly by preventing the release of GABA from the neurones mediating recurrent inhibition. The latter effect could only be responsible for the <u>initiation</u> of firing in my experiments if there was sufficient excitatory synaptic input so

that the main events which prevented cell firing were the recurrent (GABA-mediated) I.P.S.P.s. The available evidence is not conclusive but appears to favour a postsynaptic action of ACh in this situation. This is not to say that disinhibition may not contribute to the excitatory effect of ACh. The ACh-induced firing is smooth and sustained, which suggests that it is postsynaptically mediated due to "m" channel closure. It only becomes somewhat erratic when picrotoxin is added. This is probably because there may be some recurrent inhibition still to block. The observation left to explain is that picrotoxin-induced firing by itself does not interfere with responses to 5-HT. When picrotoxin is added to ACh-induced firing, the overall balance between synaptic excitation and inhibition may be shifted towards excitation, thus interfering with 5-HT-induced inhibitions; hence the firing in the presence of ACh and picrotoxin cannot be compared with the control (ACh-maintained firing). In the case of picrotoxin-induced firing, an attempt was made only to match firing rates; this simpler experimental design may leave less scope for physiological antagonism.

In conclusion, picrotoxin does not specifically interact with 5-HT-induced responses of CA_1 pyramidal cells but only with ACh-induced firing in a physiological rather than pharmacological manner. This emphasizes the point that those who have suggested that picrotoxin blocks responses to 5-HT have not demonstrated any selectivity relative to other monoamine transmitter candidates. The finding that picrotoxin application to a cell which had its firing

maintained by ACh did not reduce the effectiveness of the DOM-induced inhibitory responses, nor alter the effectiveness of DOM when the maintenance of firing was switched from ACh to picrotoxin alone, suggests that DOM is probably not acting by a direct or indirect action on GABA receptors. Also the possibility that DOM could alter the release of GABA is unlikely because DOM did not activate silent CA_1 cells. Unfortunately, due to the probable nonselective nature of the effect of picrotoxin on responses to 5-HT when cell firing is maintained by ACh, this provides no information about whether or not the effects of DOM are mediated by an action on 5-HT receptors, but only that a difference may exist between a number of factors which determine the amplitude and duration of the responses to 5-HT and DOM.

It is surprising that fluoxetine (1-3 mg/kg), the 5-HT-selective uptake inhibitor, failed to increase the duration of the inhibition of hippocampal CA_1 cells caused by pulses of iontophoretically applied 5-HT. The result is in marked contrast to that seen with 1 mg/kg fluoxetine on cortical cells (Jones and Broadbent, 1982). A similar failure of fluoxetine to prolong responses to 5-HT has been reported for CA_3 cells by de Montigny et al. (1980), who used up to 10 mg/kg of fluoxetine; in contrast, desipramine in the same study prolonged responses to NA. These authors concluded that on these cells 5-HT uptake by serotonergic terminals does not play a significant role in terminating the action of iontophoretically applied 5-HT acting at the cell soma. Support for this conclusion comes from the finding that

5-HT terminals are much more abundant in the stratum radiatum than in the stratum pyramidale (Azmitia and Segal, 1978).

3. Is there a 5-HT receptor on CA_1 cells?

Where receptors have not been chemically isolated, they are normally inferred on the basis of several criteria (adapted from Rang 1973):

1) Drugs or transmitters affect the tissue in low concentrations, and the response is concentration-dependent.
2) Small changes in the structure of the agonist greatly alter its potency; stereoselectivity of an agonist is often present.
3) The response shows a clear maximum.
4) The existence of selective antagonists.
5) Often receptor-mediated responses show desensitization after exposure to large concentrations of the agonist.
6) The development of denervation supersensitivity.

All of these criteria would not have to be fulfilled for a response to be considered to be receptor-mediated. For the inhibitory response of CA_1 cells to 5-HT, few of these criteria have been met. The first criterion has been in the sense that the size and duration of the response to 5-HT is clearly dependent on the amount that is ejected from the electrode. The currents required are no larger than those which produce inhibition at other sites in the brain, and perhaps lower on average than those required to produce excitation of FMN's (which is clearly receptor-mediated). However, inhibition of

of CA_1 cells is caused by only large amounts of 5-HT (100 μM) when tested *in vitro* (Segal, 1980). The second criterion is presumably met by the finding of Segal (1975) that 5-HIAA (the main metabolite of 5-HT) produces excitation of CA_1 cells *in vivo*, and that 5-MeoT applied with the same current or currents that produced similar initial inhibitions had inhibitory effects which were much longer lasting than were those to 5-HT. 5-HTP was much weaker than 5-HT in terms of current ejected. The third criterion can only be met when intracellular recordings are obtained from CA_1 cells. Both the bath and iontophoretic application of 5-HT *in vitro* produced a hyperpolarization and decrease in input resistance which reached a maximum (Jahnsen, 1980; Segal, 1980). Results in this thesis suggest that the fourth and fifth criteria are not met. Of course if the response is receptor-mediated, an antagonist may one day be discovered. Jahnsen (1980) and Segal (1980) found comparable inhibitory responses of CA_1 pyramidal cells to 5-HT *in vitro*. However, Segal reported desensitization of the response, while Jahnsen could find none. It should be noted that the time course of the responses recorded were substantially slower than those seen *in vivo*.

Another criterion often demonstrated by receptor-mediated events is the development of supersensitivity to the action of 5-HT which often occurs after denervation with the 5-HT neurotoxin 5,7-DHT or the 5-HT synthesis inhibitor PCPA (Wang et al., 1979; Menkes et al.,

1980). The effects of denervation of serotonergic innervation of hippocampal CA_1 cells does not appear to have been tested in vivo. However, the response of hippocampal CA_3 cells to 5-HT does not become supersensitive after denervation (de Montigny et al., 1980). It is not clear whether this is also the case with cells in the CA_1 region. Not only was the initial responsiveness of the CA_3 cells to 5-HT not increased, but also the response was not prolonged (the presynaptic type of supersensitivity). Only the presynaptic type of supersensitivity to NA occurred after 6-hydroxydopamine. These results stand in marked contrast to those obtained in the ventral LGN, amygdala and facial motor nucleus (Wang et al., 1979; Menkes et al., 1980) and the effect of chronic treatment with antidepressants on CA_3 pyramidal cells (de Montigny and Aghajanian, 1978).

Iontophoretically applied DOM was effective both as an inhibitor of the ACh or GLU-maintained firing of hippocampal CA_1 cells and in reducing the response of these cells to pulses of ACh or GLU. Whilst no attempt was made to determine an $I.T._{50}$ value for this effect, DOM was clearly less potent than 5-HT (in terms of ejecting current value) but otherwise responses to DOM appeared similar to those of LSD or D-amphetamine. As the transport number of DOM was not determined the significance of this observation remains obscure.

In conclusion, using pharmacological methods it did not prove possible to assess the role of 5-HT receptor stimulation in the effects of DOM on hippocampal CA_1 cells. It is possible (though

unlikely) that the antagonists evaluated on CA_1 cell responses to 5-HT may prevent the inhibition caused by DOM. If this occurred, then a mechanism for the effects of DOM, independent of 5-HT receptor stimulation, would have to be proposed.

4. Possible involvement of adrenoceptors in the action of DOM

Szabadi (1979) has reviewed the findings of experiments in which single unit recordings of the inhibitory and excitatory actions of catecholamines have been made *in vivo*. In this review it was concluded that the majority of *in vivo* studies in which antagonists have been used support the generalization that excitatory responses in the central nervous system are mediated by α-adrenoceptors, while inhibitory responses are media-ted by β-adrenoceptors. Aghajanian and Rogawski (1983) have refined this generalization by including results of more recent studies. It has been found that α_1-adrenoceptors mediate NA-induced excitations of LGN, DR, and FMN's, while α_2-adrenoceptors have been shown to mediate the inhibitory responses of LC neurones to NA.

As reported by Biscoe and Straughan (1966) and Segal and Bloom (1974), in the present study NA had an inhibitory effect on CA_1 pyramidal cells which was not prevented by the α-adrenoceptor antagonist phentolamine. This result is also in keeping with that of Bevan et al. (1977) who found that phentolamine and phenoxybenzamine would selectively prevent only the excitatory effects of α_1-agonists on cortical cells, but not inhibitory effects when they occurred.

D-amphetamine application produced a response which was similar to that reported by Segal and Bloom (1974). The action of D-amphetamine is presumably indirectly mediated (Burn and Rand, 1958). The finding that the actions of D-amphetamine and DOM were unaffected by phentolamine suggests that these effects were not mediated by α_1- or α_2-adrenoceptors. Both DOM and D-amphetamine lack the β-OH group which is required for α-agonist activity (Weiner, 1985).

Sotalol, the β-adrenoceptor antagonist which has the least local anaesthetic action, produced a modest overall (15-20%) reduction in the size of the inhibitory response to NA at currents which often had little effect on control baseline firing. This result is in contrast to the "complete" blockade reported by Segal and Bloom (1974), although they reported no actual measurements. Higher ejection currents of sotalol depressed baseline control firing rates, but when this was compensated for by using measurement method (c), the size of the responses to NA were still not reduced by more than 15-20%. If the altered firing pattern and slight excitatory effect of low currents of sotalol is taken into consideration, the maximum antagonism achieved was not large. The results of limited control studies with 5-HT suggested that the effect of sotalol may be selective for the response to NA. However, the time course of the response to 5-HT was clearly different from that of NA and so 5-HT may not be the most appropriate agonist to use as a control. Intuitively, it seems that an inhibitory response which lasts longer than another would be more sensitive to interruption by an irregular excitatory

input than would a short-lasting inhibitory response. The finding that the effects of D-amphetamine and DOM were not even slightly reduced by sotalol is not surprising in the light of the requirements for β-agonist activity of the phenethylamine structure; neither DOM nor D-amphetamine have a large group on their amino-terminals (Weiner, 1985). The failure of trifluoperazine or chlorpromazine to antagonize the effect of DOM suggests that DA receptor stimulation probably does not cause these responses. DA is reportedly only a weak agonist on CA_1 pyramidal cells (Segal, 1974).

In conclusion, the results of these studies with α- and β-adrenoceptor antagonists lend no support to the concept that the ability of DOM or the indirectly acting D-amphetamine to suppress hippocampal CA_1 cell firing may be mediated by an action on adrenoceptors. These experiments have not ruled out the possibility that D-amphetamine acts by releasing DA or 5-HT from sources which are remote from the cell soma, or that DOM acts directly or indirectly on receptors for 5-HT.

5. The influence of blood pressure alteration on CA_1 cell firing

There was a direct relationship between the direction of change of the firing of hippocampal CA_1 cells and any alteration in blood pressure caused by drug injection by the i.v. route. By contrast, there was an inverse relationship between the direction of blood pressure change and the firing of LC neurones (Persson and Svensson,

1981; this thesis Appendix II). As the LC may well provide a tonic inhibitory input to CA_1 cells (Segal and Bloom, 1974), this is suggestive that an indirect mechanism induced by blood pressure changes, mediated by the LC, may cause the responses recorded in area CA_1. Injection of methacholine i.v., which lowers the blood pressure, increases the firing of LC cells which may then inhibit CA_1 cell firing. Similarly, i.v. NA or DOM increases the blood pressure, which inhibits LC firing; this may lead to an excitation of CA_1 cells. The results of these studies are discussed further in Appendix II.

B. Experiments with DOM on dorsal raphé neurones

1. The direct application of DOM

The results of this study indicate that the prototype synthetic phenethylamine hallucinogen DOM, when applied directly to DR neurones, is not a potent inhibitor of firing rate. The ability to inhibit DR units is a property which is shared by proposed 5-HT autoreceptor agonists such as LSD and 5-MeoDMT (de Montigny and Aghajanian, 1977). Mescaline, a natural phenethylamine also did not inhibit DR units as previously reported (Haigler and Aghajanian, 1973). D-amphetamine, on the other hand, profoundly depressed the DR firing rate when applied directly to the cells. The simplest explanation for this effect would invoke the ability of D-amphetamine to release 5-HT from 5-HT-containing terminals which have been proposed to exist near the DR neurones (Wang and Aghajanian, 1978; Geyer et al., 1978). However the possibility that D-amphetamine releases DA from DA-containing

terminals has not been ruled out. Since DOM is much less effective than 5-HT or D-amphetamine, it appears not to act by release of 5-HT thus confirming the results of other investigations (see Introduction). Although DOM has a greater structural similarity to D-amphetamine than does mescaline (DOM has an α-methyl group, see Fig. 1), methoxylation of the ring structure of amphetamines decreases their ability to release monoamines (Iversen, 1971; Paton and Pasternak, 1974; Biel and Bopp, 1975).

The receptors found on DR cell bodies have characteristics of the 5-HT_{1A} binding site, and DR cells are potently inhibited by the 5-HT_{1A} ligand 8-OH-DPAT (de Montigny et al., 1984; Vergé et al., 1985). Those receptors found on the DR terminals appear, however, to be different from those on the cell bodies (Vergé et al., 1985). Consequently, although DOM is not a potent inhibitory agonist at the cell bodies of DR neurones, it would be rash to conclude that mescaline and DOM should be ineffective in decreasing 5-HT release by a presynaptic feedback action on raphé terminals elsewhere.

2. The effect of DOM given by the intravenous route

The finding that DOM, when given by the i.v. route, often produced a suppression of DR firing (where the cell was not lost) is a difficult one to explain with any certainty. There are several possibilities which can be advanced. Aghajanian et al. (1978) have shown that noxious stimulation delivered by sciatic (peripheral) nerve stimulation, at low frequencies, produced a short pause in DR neurone firing. If acute blood pressure increases represent a noxious

stimulus akin to peripheral nerve (sciatic) stimulation, then it is possible that a short duration cessation of firing may occur mediated by afferent spinal pathways yet to be identified. However, on i.v. injection of DOM, suppression of firing was usually long-lasting, that is for the duration of the blood pressure alteration (12 minutes in Figure 19). Another possibility is that changes in blood pressure somehow directly affect the firing of DR neurones. This may be the case for firing of the NA-containing LC cells, as the firing of LC neurones is phase-locked to the heart beat, unlike that of DR neurones which is not (Morilak et al., 1985). As iontophoretically applied NA excites DR neurones, and these cells are innervated by the LC, their firing rate may be modulated or maintained by the influence of LC neurones (Baraban and Aghajanian, 1979; Vandermaelen and Aghajanian, 1983); but other groups do not agree with this proposal (Heym et al., 1981; Trulson and Crisp, 1984). DOM, or any drug which increases the blood pressure should inhibit LC firing (Persson and Svensson, 1981); this may remove a tonic source of excitation usually provided to DR neurones. The lack of effect of iontophoretically applied DOM to DR neurones agrees in some respects with the results of Trulson et al. (1981) who found that i.v. DOM (in the conscious cat) did not inhibit DR units. It is probable that vasomotor changes evoked by i.v. drugs are more severe in the anaesthetized animal. As directly applied D-amphetamine inhibited DR cells, this suggests that

the variable responses, or excitation (Foote et al., 1969), seen with i.v. administration may be caused by a number of indirect mechanisms.

The concept that the various raphé nuclei are not the main site of action of hallucinogens is supported by the fact that phenethylamines appear to have little agonist potency at the receptor type expressed on their cell bodies. It was thought that hallucinogens in general were less potent at postsynaptic 5-HT receptors (Haigler and Aghajanian, 1974a; de Montigny and Aghajanian, 1977). This may be the case for indolealkylamines and LSD acting at receptors mediating inhibition, however DOM and presumably mescaline may be more potent inhibitors of the target cells of DR neurones (where 5-HT produces inhibition) than of DR cell firing. In support of an action at target cells, it has recently been reported that most hallucinogens bind with a high affinity to 5-HT_2 postsynaptic sites (Glennon et al., 1985). These may be the sites that mediate the excitatory responses caused by DOM on FMN's (Penington and Reiffenstein, 1985; and Section C below).

Although it has been estimated that in the DR of the rat only one in three neurones is serotonergic (Descarries et al., 1982), the criteria used for DR cells in this study probably selected for serotonergic cells. This is because cells found in the DR reveal three types of activity when recorded extracellularly (Aghajanian et al., 1978b): (1) Slow (0.25-2.0 SPS), regular, spontaneously active cells with a large positive spike of long duration (1-2 msec); (2)

Very slow or quiescent cells, usually displaying small negative action potentials; and (3) cells with relatively high rates of discharge (i.e. 2-9 SPS), often irregular in rhythm. Only the first type of cells have been identified as being serotonergic (Wang and Aghajanian, 1977b; Aghajanian and Vandermaelen, 1982b) and only this type of cell was examined in this study. The type 2 cells may be interneurones as they are on average half the size and fire in a reciprocal fashion to 5-HT-containing cells. The type 3 cells may be the ones found to contain tyrosine hydroxylase but not dopamine β-hydroxylase, indicating that these are DA-containing neurones (Miachon et al., 1984). This work does not rule out the possibility that these cells may take up 5-HT as well and so would be difficult to demonstrate with histoflurochemical methods. In conclusion, this work has shown that DOM is clearly different from 5-HT, LSD and D-amphetamine in that it is only a weak autoreceptor (S_2) agonist at DR cells. The effects of DOM resembled those of mescaline, the natural prototype phenethylamine hallucinogen.

C. Experiments on facial motor neurones

1. The action of 5-HT, NA, DA and DOM

As originally described by McCall and Aghajanian (1979a), FMN's, when exposed to small amounts of 5-HT or NA, show greatly facilitated excitatory effects of iontophoretically applied GLU. Results of the present study extend these findings by demonstrating that under the same conditions DA and the phenethylamine hallucinogen DOM will induce FMN firing. These FMN's can be considered a useful model of other

synapses where 5-HT and DA may produce excitation. The effect of 5-HT on the FMN's themselves is likely to be physiologically relevant, as these cells receive a dense and uniform serotonergic innervation with serotonergic nerve terminals making axo-somatic and axo-dendritic synapses (Aghajanian and McCall, 1980). Whether the responses to NA and DA have a physiological role is less clear, as the innervation by NA-containing neurones is much weaker (McCall and Aghajanian (1979a) and DA-containing neurones may be non-existent (Levitt and Moore, 1979) although this problem has not yet been adequately addressed. Available evidence confirms the suggestion that the effects observed in the facial nucleus are postsynaptically generated, and not complicated by indirect effects on local interneurones. The reasoning behind this statement is two fold: first it has been reported that there are no interneurones in the nucleus (Courville, 1966; Aghajanian and McCall, 1980), and secondly when the response is recorded intracellularly, 5-HT does not depolarize the cells by acting presynaptically to reduce the tonic release of a chloride-mediated inhibitory neurotransmitter (Vandermaelen and Aghajanian, 1982b). As the effect of iontophoretically applied GLU (as well as synaptic input) is facilitated by 5-HT, it would appear that this action is mediated by a postsynaptic mechanism. However, when the effects of excitatory synaptic input are increased by 5-HT (such as the response to stimulation of the red nucleus) then 5-HT may act on the afferent

terminals to increase transmitter release. This mechanism is probably not seen under physiological conditions as axo-axonic synapses involving serotonergic fibers have not been found in the mammalian central nervous system (McCall and Aghajanian, 1979a). Whilst inhibitory interneurones appear to have been ruled out by all investigators, there may be afferent terminals which release glycine onto FMN's. Martin et al. (1977) have shown that strychnine will selectively prevent the inhibitory effect of glycine on FMN firing and also the inhibitory effect of lingual and glossopharyngeal nerve stimulation.

The finding that it was usually difficult to elicit alternating responses to NA and DA is puzzling in that it suggests that there could be cross-desensitization occurring between them, assuming the two compounds were activating the same receptor. In the periphery this has been noted to occur but DA is 1/40th as potent as NA on the α_1-adrenoceptor (Goldberg, 1972). On a spinal motor neurone preparation, which has yielded results analagous in many ways to those from the facial nucleus (White and Neuman, 1982), other groups have had the same difficulty when they attempted to alternate responses to NA and DA (Barasi and Roberts, 1977). Despite this difficulty, Barasi and Roberts (1977) concluded that DA activated a DA receptor and NA a separate receptor because α-flupenthixol blocked DA, but potentiated responses to NA. In the same study, α-flupenthixol clearly differentiated between responses to 5-HT and those to DA.

This finding has its parallel with results in this thesis as the neuroleptic chlorpromazine antagonized the DA-elicited responses of FMN's but not those caused by 5-HT. That DOM was a pure agonist at these receptors is interesting in view of the spectrum of effects noticed with hallucinogens on these cells (McCall and Aghajanian, 1980b). At very low currents, LSD sensitized the FMN's to NA and 5-HT, while at higher currents of LSD an antagonist action was noted. Similarly, with mescaline low currents had a sensitizing effect, but unlike the effect of LSD, as the amount of mescaline ejected was increased an agonist action became apparent. The indoleamine hallucinogens (e.g. 5-MeoDMT) were pure agonists. McCall and Aghajanian (1980b) noted that this spectrum of effects has often been observed with hallucinogens on peripheral "D" receptors (see Introduction, B.2).

Interestingly, it is probable that the excitatory effect of DOM may manifest itself partly in the form of involuntary facial muscle twitches. Virola snuff taken by native indians of the upper Amazon contains the hallucinogen 5-MeoDMT (and a MAOI which prevents destruction of 5-MeoDMT in the brain). This compound is a pure excitatory agonist on FMN's (McCall and Agajanian, 1980b). Schultes (1972) has reported that the most noticeable physical effect of the use of this snuff is unconscious twitching of the facial muscles.

2. The effect of 5-HT antagonists on responses to 5-HT, NA, DA and DOM

As methysergide has perhaps the lowest affinity for α_1-adrenoceptors of all "D" antagonists (Janssen, 1983; Humphrey, 1984), being 1000 fold lower than its affinity for the 5-HT_2 or "D" receptor, it is not surprising that responses to 5-HT and not NA were antagonized by methysergide. It can be inferred that as very low currents of methysergide completely and reversibly prevented the excitatory response of FMN's to DOM that this effect would not be due to an α_1-antagonist action. Indeed, responses to DOM were prevented by methysergide when those to NA were unaffected. On the basis of these findings these data suggest quite strongly that DOM does not excite FMN's by an action on receptors for NA.

In contrast, the selectivity of methysergide on responses to 5-HT and DA is not nearly so clear. As methysergide has seldom, if ever, been tested for its ability to discriminate between neural excitatory responses to 5-HT and DA until this work was carried out, the only precedents are the results of binding studies. Both Whitaker and Seeman (1977) and Janssen (1983) have reported that the binding of methysergide hardly discriminates at all between DA and 5-HT binding sites. This is reflected in the results seen in the present study as methysergide failed to discriminate between responses to 5-HT and DA. As methysergide antagonized responses to 5-HT but not those to NA, this can be considered as further evidence (see Section 3) that the

receptors for NA and DA are not identical.

It should be borne in mind that in order to prove that a receptor characterized by radioligand binding studies is the same as one studied by observing a physiological response, several requirements must be met. First a comparison of pA_2 values is useful, but an extensive analysis of both agonist and antagonist must be performed in both systems, bearing in mind that different tissue barriers to the receptors may be present (Furchgott, 1979). Nevertheless, almost all the data available in each system suggests that the 5-HT_2 binding site, the 5-HT_2 receptor, the "D" receptor and the S_1 receptor (FMN's) could be one and the same. This concept would be further strengthened if ketanserin, the selective 5-HT_2 receptor antagonist and ligand, was shown to antagonize the effect of 5-HT on FMN's. This was the case in these experiments. However, when tested with NA and DA as the control agonists, ketanserin could not discriminate between 5-HT and NA or DA. While it is relatively difficult to choose an exactly appropriate (selective) concentration of antagonist with some drugs using microiontophoresis, ketanserin's lack of selectivity with respect to other putative neurotransmitter receptors is well known (Janssen, 1983). The selectivity of ketanserin is between 5-HT receptors; this underscores the fact that a truely specific 5-HT_2 antagonist has not yet been found.

3. The effect of chlorpromazine on responses to 5-HT, DA and DOM

In order to show that the response to 5-HT was due to an action

on 5-HT and not DA receptors, the use of 5-HT antagonists alone is inadequate. A selective DA receptor antagonist was sought. Somewhat surprisingly, chlorpromazine proved to be such an agent. The initial reasons for choosing it were: it is soluble, charged at pH 4 and thus can be used without the addition of organic acids; second, it had been shown not to antagonize responses of FMN's to NA and 5-HT at sub-local anaesthetic concentrations (McCall and Aghajanian 1980a) or the excitatory response to NA of randomly encountered brainstem neurones (Boakes et al., 1979). Interestingly, chlorpromazine does not prevent DA-mediated inhibitory responses of substantia nigra neurones (Aghajanian and Bunney, 1973). This is not because chlorpromazine produces local anaesthetic effects at concentrations lower than those required to reach an antagonist concentration, because chlorpromazine only becomes a local anaesthetic at 0.1-1 μM. Both the antagonism of DA receptors and therapeutic levels of the drug occur in the low nM range (Seeman, 1977). It would be interesting to check for the presence of dopaminergic innervation of the facial motor nucleus. As these cells control the movement of the facial muscles and respond with excitation to DA application which is selectively prevented by chlorpromazine, this may serve as a model of the Buccolingual masticatory syndrome (one of the Tardive Dyskinesias). This condition of involuntary facial twitching is seen in patients who have been exposed to chronic treatment with neuroleptics and is hypothesized to be due to the development of super-sensitive DA receptors as a result

of their blockade (Simpson et al., 1981).

To show that specific receptors for DA do not mediate the link between the simulus DOM and the excitatory response it produces, it is necessary to show that the response to DOM is not reduced by sub-local anaesthetic amounts of chlorpromazine (which antagonized responses to DA). This is essentially what occurred when responses to DOM were alternated with those to 5-HT as control agonist. However some difficulty was encountered when an attempt was made to alternate responses to DOM and DA: one or the other response was often lost. This was reminiscent of the failure to alternate reliably responses to NA and DA. In two successful experiments where the effect of DOM and DA was compared, the response to DA was readily and reversibly antagonized (with no apparent local anaesthetic effect), but the response to DOM was depressed either irreversibly or showed a long recovery phase, much longer than the time course of the recovery of the response to DA. A somewhat speculative explanation for this puzzling observation may lie with a 'selective' local anaesthetic interaction. Where local anaesthetic effects as a result of chlorpromazine application occurred, responses to 5-HT and DA recovered much quicker than did responses to DOM. The reason for this may lie in the relative lipophilicity of 5-HT and DA relative to chlorpromazine and DOM. Handschumacher and Vane (1967) have shown that smooth muscle cell walls act as a diffusion barrier which excludes 5-HT but not tryptamine from entering the cell. Similarly,

the DA molecule is quite polar. This is not the case for chlorpromazine or DOM (Seeman, 1977; Nichols et al., 1977). If a certain amount of chlorpromazine gets into the membrane, but not enough to cause an alteration in the action potential amplitude, the application of 5-HT or DA would not add to this effect. The addition of a lipophilic compound (DOM) may add to a non-specific membrane disordering effect of chlorpromazine; the action potential amplitude may recover somewhat between applications of DOM, enough to allow a polar compound (i.e. DA or 5-HT) to elicit a normal sized action potential. In this way an anaesthetic effect could be noted on DOM-elicited action potentials but not on 5-HT or DA-elicited spikes. Alternatively, DOM may be a mixed agonist and also have some weak DA agonist activity as suggested for DOM and mescaline on the basis of behavioural studies (Trulson et al., 1977; Trulson et al., 1983a). A clearer resolution of this point requires knowledge of absolute agonist and antagonist concentrations which cannot be achieved in vivo.

In this work no attempt has been made to rule out the possibility of indirectly mediated responses to DOM on central neurones for several reasons:

1. A considerable amount of evidence discussed in sections B2, C and D of the Introduction suggests that, unlike PMA, MDA or D-amphetamine, DOM is not an indirectly acting agent.
2. While the indirectly acting agent D-amphetamine profoundly

inhibited the firing of putative serotonergic cell bodies in the DR nucleus, DOM was very weak in this respect and mescaline was inactive.

3. The effectiveness of methods to deplete monoamines and cause terminal degeneration in vivo always carries with it an uncertainty of how complete a denervation is achieved. Consequently it was decided that this approach may not provide a clear-cut answer and rule out any indirectly-mediated actions of DOM.

D. Summary and concluding remarks

The object of this thesis was to provide some evidence which has a bearing on the proposal that the phenethylamine hallucinogen 2-5,dimethoxy-4-methylamphetamine (DOM) (as a prototype of the class) can influence rat brain nerve cells in a similar manner and by the same receptors as does the putative neurotransmitter serotonin (5-HT). Results of this thesis indicate that DOM produces an inhibition of hippocampal CA_1 cell firing rate, but is not as effective in terms of ejection current as is 5-HT. On CA_1 cells (the S_3 site, Aghajanian, 1981) the effects of DOM resemble those of other classes of hallucinogens (i.e. LSD and the indolealkylamines, Haigler and Aghajanian, 1974a; de Montigny and Aghajanian, 1977). However, LSD and the indolealkylamines are potent inhibitors of spontaneously active serotonergic dorsal raphé neurones (DR); results of this thesis indicate that the phenethylamine hallucinogen DOM, unlike the

indirectly acting agent D-amphetamine, is not. This result is in general agreement with those of Haigler and Aghajanian (1973) who found that the natural prototype phenethylamine hallucinogen mescaline was ineffective at slowing the firing of DR neurones. In other words, indolealkylamine hallucinogens and LSD show a preferential affinity for presynaptic S_2 receptors (perhaps $5\text{-}HT_{1A}$ sites) but this is not the case for the phenethylamine group. Behavioural, binding, and classical pharmacological studies indicate that DOM may be a $5\text{-}HT_2$ agonist (Introduction F; Dyer, 1976; Glennon et al., 1985). $5\text{-}HT_2$ receptors may mediate 5-HT-induced excitation in the central nervous system (Davies et al., 1985); results of this thesis show that application of DOM excited facial motor neurones (FMN's) in a similar fashion to 5-HT.

On hippocampal CA_1 cells representative drugs from each class of antagonists ("D", "M", $5\text{-}HT_1$ and $5\text{-}HT_2$) failed to prevent the inhibitory effect of 5-HT. In this context it should be noted that binding studies usually cannot distinguish between agonist and antagonist drugs. This result is in keeping with the generalization that inhibitory responses to 5-HT in the central nervous system are usually not prevented by any known 5-HT antagonist (Aghajanian, 1981). Hence it was not possible to obtain antagonist data on 5-HT receptors which may or may not mediate the response to DOM of hippocampal CA_1 pyramids.

The DOM-induced excitation of FMN's however was selectively antagonized by methysergide, as were responses to 5-HT (McCall and

Aghajanian, 1979a). Although data presented in this thesis suggests that the DOM-elicited responses were not mediated by an action on DA receptors, a local anaesthetic effect of chlorpromazine during responses to DOM prevented the conclusive ruling out of some degree of mixed agonist action of DOM on receptors to both DA and 5-HT. However, the demonstration that DA also facilitates the firing of FMN's probably by an interaction with its own specific receptors, was unexpected.

As the 5-HT_2 selective antagonist ketanserin prevented the 5-HT-mediated excitation of FMN's, this is supportive of the concept that all hallucinogens may interact with 5-HT_2 type receptors in the central nervous system. An impressive correlation exists between the affinity of fifteen hallucinogens for 5-HT_2 binding sites and the threshold dose required for hallucinogensis (Glennon et al., 1985). An interaction with 5-HT_2 receptors may underlie or contribute to the overall mechanism of action of hallucinogenic drugs.

After examining the effects of DOM upon hippocampal CA_1 cells, DR neurones and FMN's the results imply that the net effect of DOM administration (discounting indirectly-mediated actions) is not disinhibition of the higher centres, at least not those innervated by the DR nucleus, as the DR is not inhibited. The direct effect of DOM on the limbic and higher centres is probably an inhibitory one. Where 5-HT is excitatory (e.g. FMN's and areas in the brainstem and frontal cortex), DOM should also exert an excitatory influence, one which

should not be counteracted by the removal of an excitatory tone from the DR or whichever serotonergic nucleus is responsible for their innervation.

Finally, the antagonism of the behavioural effects of hallucinogens in animals by 5-HT (5-HT_2) antagonists (Browne and Ho, 1975; Commissaris et al., 1981; Colpaert et al., 1982; Glennon et al., 1983; Heym et al., 1984) is also likely due to actions at sites where 5-HT produces excitation. Moreover, the behavioural effects of phenethylamine hallucinogens such as DOM and mescaline are differentially (more effectively) antagonized by 5-HT antagonists when compared to the effects of the antagonists on the indolealkylamine group (Browne and Ho, 1975; Commissaris et al., 1981). Taken together, these results suggest that the main groups of hallucinogenic molecules have dissimilar actions on different 5-HT receptor subtypes in addition to possible effects on receptors for different putative neurotransmitters.

CHAPTER V

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APPENDIX I

Results from Parry and Reiffenstein (unpublished)

Initial studies have compared the effects of 2,5-dimethoxy-4-methylamphetamine (DOM) and para-methoxyamphetamine (PMA) to those of D-amphetamine and 5-hydroxytryptamine (5-HT), since these seemed representative of the effects seen in the behavioural studies - one appearing mainly tryptaminergic, the other possibly having dual effects. So far mostly superficial cerebral or cerebellar cells have been examined, with large stable action potentials, rather than midbrain neurones. The following summary table describes the results of various drug applications.

TABLE A Drug effects on neurone firing rates

DRUG	INCREASE	DECREASE	NO CHANGE	TOTAL
5-HT	28 (29)*	47 (49)	21 (22)	96
D-AMPHETAMINE	8 (9)	73 (82)	8 (9)	89
DOM	17 (25)	31 (45)	21 (30)	69
PMA	7 (22)	17 (56)	7 (22)	31

*Number of neurones (% of total tested)

For neurones which could be recorded for periods sufficient to make multiple comparisons, the following correlation data were obtained (Tables 2-5). A high correlation in direction of effects of 2 drugs is indicated by relatively high values in the diagonal from upper-left to lower right.

TABLE B Correlation between 5-HT and DOM effects on neurone firing rates (number of neurones responding)

		INCREASE	DOM DECREASE	NO CHANGE
	Increase	9	2	3
5-HT	Decrease	5	13	2
	No change	3	0	9

TABLE C Correlation between D-amphetamine and DOM effects on neurone firing rate

		INCREASE	DOM DECREASE	NO CHANGE
	Increase	2	8	0
D-AMPHETAMINE	Decrease	10	8	13
	No change	1	2	1

TABLE D Correlation between 5-HT and PMA effects on neurone firing rates

		INCREASE	PMA DECREASE	NO CHANGE
	Increase	6	1	2
5-HT	Decrease	2	13	2
	No change	1	0	4

TABLE E Correlation between PMA and D-amphetamine effects on neurone firing rates

		PMA INCREASE	PMA DECREASE	NO CHANGE
D-AMPHETAMINE	Increase	0	0	0
	Decrease	7	17	7
	No change	0	0	0

D-Amphetamine was generally a very potent (in terms of ejecting current) inhibitor of most cells studied, and the effect was rapid in onset. 5-HT on the other hand had a more variable effect, and while about half of the neurones were depressed, one-quarter of the total were excited. The onset of effect was also generally slower than with D-amphetamine. 5-HT, DOM and PMA all required much more current than D-amphetamine to exert their effects (currents up to 200 nA were used). Despite their D-amphetamine structure, PMA and DOM were clearly similar to 5-HT rather than D-amphetamine, in terms of potency, rate of onset, variability in effects, and correlation of direction of effects in the same neurone. Generally, where 5-HT and D-amphetamine had opposite effects, PMA and DOM were similar to 5-HT (Tables 2-5). So far, effects of direct iontophoretic application of PMA and DOM correlate with their 5-HT-like effects seen after systemic administration of these chemicals. At this stage it is not possible to say whether this similarity is a direct effect on 5-HT receptors, or occurs indirectly by release of 5-HT. In the future methysergide will be used to see if D-amphetamine, PMA and DOM effects can be further separated by pharmacological antagonism.

APPENDIX II

Drug-Induced Blood Pressure Changes Indirectly Alter the Firing Rate of the Locus Coeruleus and Hippocampal CA_1 Cells

This work describes an inverse relationship between drug-induced alterations in blood pressure and the firing rate of the locus coeruleus and subcoeruleus (LC); also described is a direct relationship between blood pressure changes and the firing rate of hippocampal CA_1 cells. Drugs which alter the blood pressure by only peripheral actions will alter LC firing. This complicates studies of LC function, and of the function of the projection areas of the LC, if drugs which alter blood pressure are given (i.v.). While the LC may or may not be a primary vasomotor center, reflexes affecting the sympathetic system inevitably affect LC firing. Surprisingly, this point seems to have been frequently overlooked in recent studies of the central actions of various drugs.

In the course of studying the responses of LC neurons to i.v. applications of cyclobenzaprine (CBZ) and other drugs, it became apparent that there is conflicting evidence in the literature regarding the control of the firing rate of these cells. A large array of drugs increase LC cell firing when intravenously applied, including both α_1- and α_2-selective antagonists such as prazosin (Dresse and Scuvee-Moreau, 1981) and yohimbine (Penington and Reiffenstein, 1982). Cedarbaum and Aghajanian (1977) have characterized adrenoceptors on LC cells by qualitative iontophoretic

application of adrenoceptor agonists and antagonists to single LC neurons. They concluded that the receptors on LC cell bodies were of the α_2-type. This has been confirmed in the LC slice preparation by Egan et al. (1983): the hyperpolarizations mediated by noradrenaline (NA) were prevented by α_2-antagonists but not α_1- or β_1-antagonists.

In view of the reports that blood volume alteration changes central NA levels and alters LC firing, (Persson and Svensson, 1981) an attempt was made to ascertain if changes in LC firing could be correlated with blood pressure changes induced by the i.v. application of drugs. The firing rate in response to blood pressure alteration of hippocampal CA_1 pyramidal cells, a projection site of the LC, was also studied.

Male Sprague Dawley rats (200-300 g) were anaesthetized with urethane (1.2-1.5 g/kg, i.p.). Cannulae were placed in the femoral vein for i.v. administration of drugs, and in the femoral artery to monitor blood pressure with a Statham transducer. Blood pressure of these anaesthetized animals was commonly between 70 and 100 mm Hg. The rats were placed in a Narishige SR5 stereotaxic apparatus with the top of the tooth bar level with the interaural line. A burr hole was drilled in the skull over the LC (1.1 mm L, 1.1 mm P to λ and 7.1-7.3 mm ventral to the brain surface) or hippocampus and the lateral sinus was ripped (and the bleeding stopped with bone wax) or pushed aside. Neurones in the LC were identified by previously reported criteria

(Cedarbaum and Aghajanian, 1977). The method employed for recording from hippocampal pyramidal cells has been described in the Methods section of this thesis.

In this study, single barrel glass micropipette electrodes were used for recording unit activity, and contained 0.5 M sodium acetate and 2% pontamine sky-blue dye. Tip diameters were in the region of 1-3 μm and the electrodes commonly had resistances of 5-10 MΩ. Signals were displayed on an oscilloscope, and single spikes discriminated by height were counted by an integrating rate meter, which displayed the cumulative count over the preceeding 2.1 sec on a chart recorder. Only one cell was studied in each animal, and the recording site was marked by passing a 10 μA cathodal current through the recording pipette for 10 min at the end of the recording session, depositing a blue spot. The brain was then fixed by intracardiac perfusion of formaldehyde, removed, and left in fixative overnight. 50 μm thick sections were cut in a Slee freezing microtome, and sections showing the dye marks were stained with safranin red.

Unperturbed LC and SC cells were commonly silent under urethane, but occasionally showed brief periods of activation at 5 spikes per sec (SPS). These periods of firing corresponded in time to periods of spontaneous reductions of blood pressure (Appendix (App) Fig. 1). After the i.v. application of 0.5-3 mg/kg CBZ (a tricyclic derivative which lowers blood pressure), the firing rate of LC cells increased

to 10-15 SPS for the duration of the blood pressure fall (30-45 min). This occurred in all 12 animals (App. Fig. 3).

Bilateral vagotomy (n=4) or atropine methylnitrate did not affect the hypotensive effect of CBZ, nor the increase in firing rate of LC cells. Methacholine (10 μg/kg, which should not enter the brain) caused a brief reduction in blood pressure, and increased the cell firing rate for the same time period (n=4). Atropine methylnitrate blocked both the methacholine-induced vasodepression and the increase in firing rate. The selective α_1-adrenoceptor antagonist prazosin (0.5 mg/kg) caused a vasodepression which was long lasting, and this was paralleled by an increase in LC firing rate.

In 11 experiments yohimbine (0.25-0.5 mg/kg) increased LC cell firing rate. Blood pressure was monitored in 2 of these experiments, and showed that the dose range employed caused a short duration vasodepression (10-15 min) which paralleled the change in firing rate (n=2).

In 6 experiments i.v. clonidine (9-75 μg/kg) decreased spontaneous or CBZ-induced firing for a period which was dose dependent (2-10 min for 9-35 μg/kg). The acute blood pressure response to these doses of clonidine was pressor (see also Hoefke, 1980) and the LC firing was depressed for a similar time to that of the blood pressure elevation.

Cells reported in this section of the study were shown by histology to be in the ventral LC or subcoeruleus. Cells which were more

dorsal and anterior showed only a moderate increase in rate (2-5 SPS) but maintained the elevated rate for a similar period.

There appeared to be a close temporal correlation between the regularity of the firing rate of hippocampal CA_1 cells and the constancy of the blood pressure. In each instance where the synthetic opiate methadone (n=16) (given i.v.) decreased the blood pressure, there was a time-linked decrease in the firing rate of the single hippocampal pyramidal cell (App. Fig. 4). There was also a late onset inhibition after i.v. drug (not shown), which may have been due to a direct effect of the drug on these cells. The effect of other drugs which decreased the blood pressure (methacholine) and those which raised it (DOM and NA) have been discussed in the main body of this thesis.

It is clear from these experiments that the firing rate of the LC and hippocampus in the anaesthetized rat is strongly affected by even small blood pressure fluctuations. The dependency of LC firing on the blood pressure was shown by the correlation between the periods of cycling activation during spontaneous blood pressure dips (App. Fig. 2), and by changes in the firing during drug-induced alteration of blood pressure. This possibility appears to have been overlooked in studies on the LC where drugs were given by the i.v. route. This effect may be especially important in the anaesthetized preparation, which has reduced vasomotor reflexes and a consistently lower blood pressure.

Increased LC firing induced by i.v. CBZ or by prazosin is likely to be simply due to the direct vasodepressor effects of these drugs resulting from the blockade of peripheral α_1-adrenoceptors. As bilateral vagotomy and the use of atropine did not alter the effects of CBZ, the effect is unlikely to be due to a direct vagal link from the heart baroreceptors. Previous studies (Ward et al., 1976) have seen no change in LC firing after atrial stretch. Interestingly, Ward et al. (1976) have reported that stimulation of the LC causes a pressor effect as has been observed in several other species (Goadsby et al., 1982; Snyder, 1975). This would suggest that firing of the LC in response to a lowering of the blood pressure is involved in restoring the original pressure. Central α_1-adrenoceptors are unlikely to contribute directly to the regulation of the LC cell firing rate, although this was proposed by Dresse and Scuvee-Moreau (1981) on the basis of the acute effects of i.v. administered drugs. Aghajanian (1982) recently reported that A5 neurones are also highly sensitive to blood pressure, and it is likely that all central noradrenergic nuclei are influenced by changes in blood pressure, and other activators of sympathetic reflexes.

The response of hippocampal CA_1 cells to iontophoretically applied opiates is an excitatory one (Segal, 1977; Fig. 5). This has been suggested to be caused by a disinhibitory action upon the CA_1 cells due to an effect of opiates which may prevent the release of GABA from the surrounding basket cells (Nicoll et al., 1980). An

excitatory response to methadone is not observed if the cell firing is maintained by acetylcholine (ACh) or glutamate (GLU); in this case the response is converted to one of inhibition (App. Fig. 5). However when methadone is given by the i.v. route, the time course of the fast inhibitory phase of the response to methadone correlates exactly with the blood pressure fall (App. Fig. 4) as was demonstrated to be the case with methacholine.

Iontophoretically applied NA inhibits CA_1 cells (Biscoe et al., 1966) as does stimulation of the LC (Segal, 1975); as drug-induced falls in blood pressure (to CBZ, methadone or methacholine) will increase the firing rate of the LC this could lead to a synaptic inhibition of CA_1 cells. Conversely, drugs which initially cause a rise in blood pressure (i.e. clonidine, D-amphetamine, DOM or NA) but do not necessarily get in to the central nervous system will inhibit the LC and may cause the CA_1 cell firing rate to increase.

In conclusion, LC and hippocampal CA_1 neurones are highly responsive to changes in peripheral blood pressure, and attempts to identify central receptors (especially in adrenergic nuclei) using the i.v. route for drug administration are likely to be misleading if peripheral vascular changes are incurred.

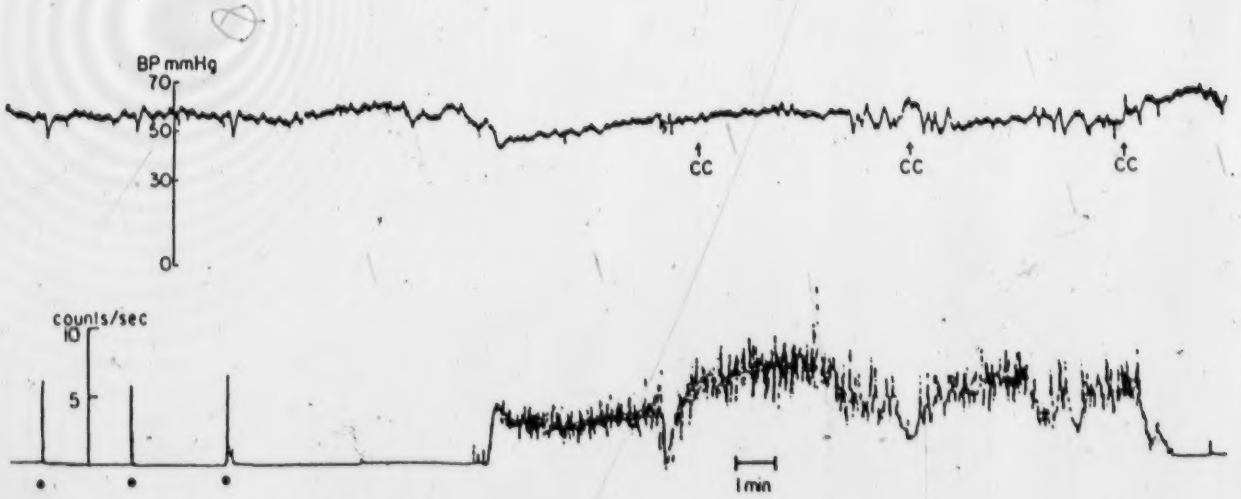

Fig. 1. Firing of a single cell in the nucleus locus coeruleus (LC) and recording of the blood pressure of an anaesthetized rat. At the circles, the rat's tail was pinched with a spring loaded clip. Note that spontaneous reductions in the blood pressure are accompanied by periods of activation of the cell firing. The labels CC refer to the clearing of the blood pressure cannula.

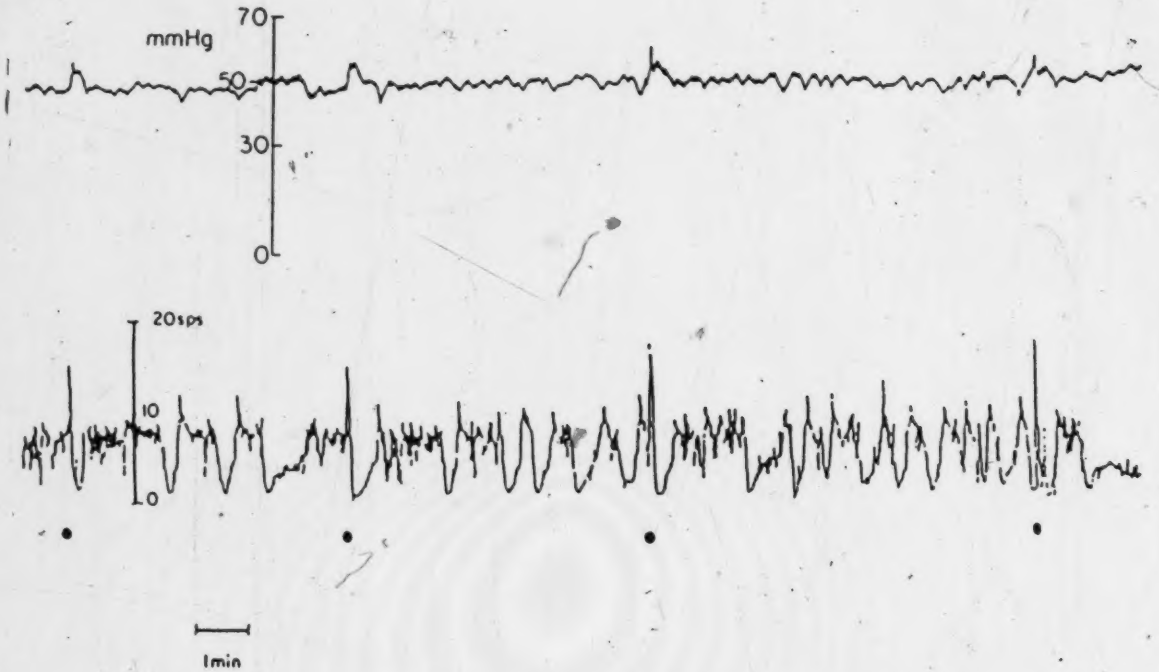

Fig. 2. Irregular firing of a locus coeruleus (LC) cell which usually coincided with a period of fluctuating blood pressure. Note that with this cell the pinching of the rat's tail resulted in a brief burst of accelerated firing, followed by a post-stimulus suppression which lasted for 20-30 sec. This period of suppression coincided temporarily with a post tail-pinch rise in blood pressure. This agrees with the observation that drug-induced changes in blood pressure are associated with an inverse influence upon LC cell firing.

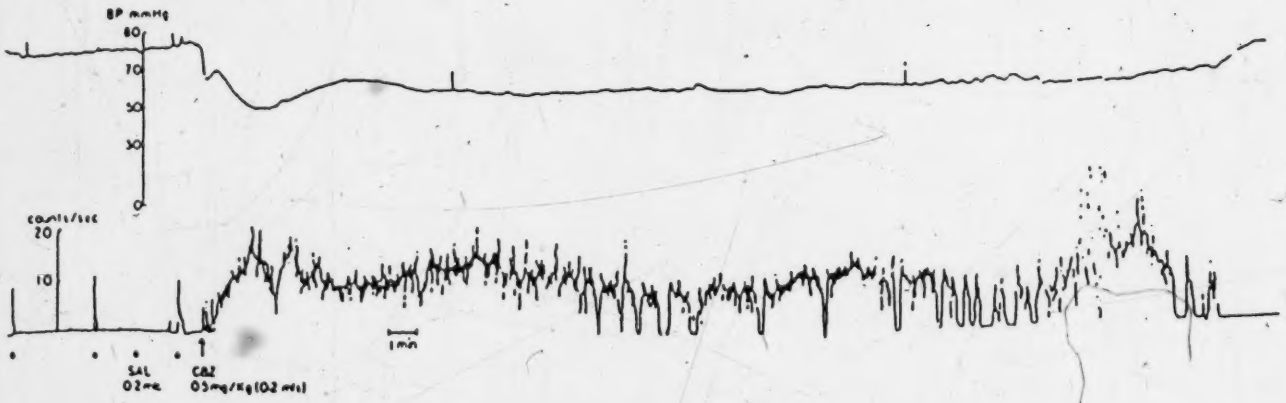

Fig. 3. Record of cell firing from a neurone in the locus coeruleus (LC) and blood pressure change upon intravenous injection of the tricyclic compound cyclobenzaprine, HCl. The injection of CBZ 0.5 mg/kg caused a profound and long lasting fall in the blood pressure, which was accompanied by a large sustained increase in the firing rate of the cell. Recovery to the usual silent state of the cell occurred only when the control level of blood pressure was re-established.

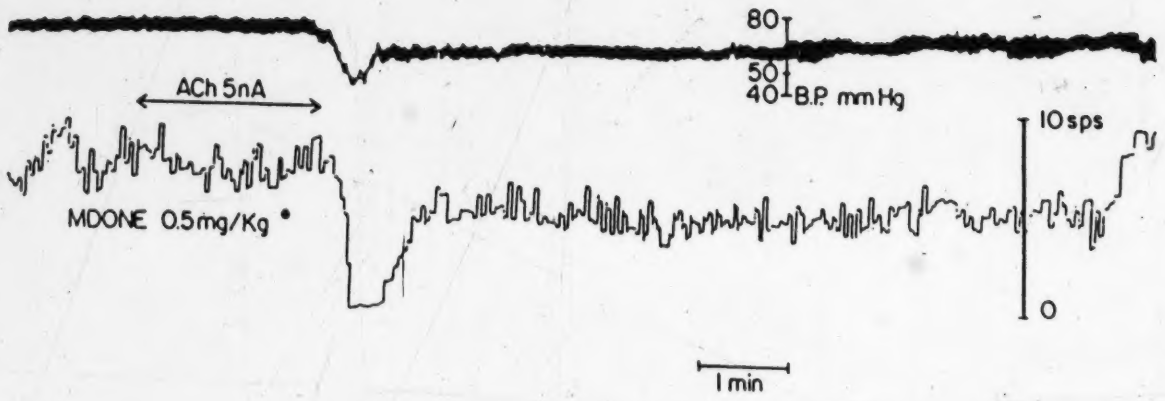

Fig. 4. Firing of a cell maintained by acetylcholine (ACh) in area CA_1 of the hippocampus. The firing rate of this cell was quite smooth, perhaps partly due to the fact that the blood pressure was stable. An intravenous dose of methadone sulphate 0.5 mg/kg (MDONE) induced a quick reduction of the blood pressure which was accompanied by the total cessation of cell firing. When about 50% of the fall had recovered, a similar proportion of the CA_1 cell firing rate also recovered. There was a direct relationship between CA_1 cell firing rate and blood pressure alteration.

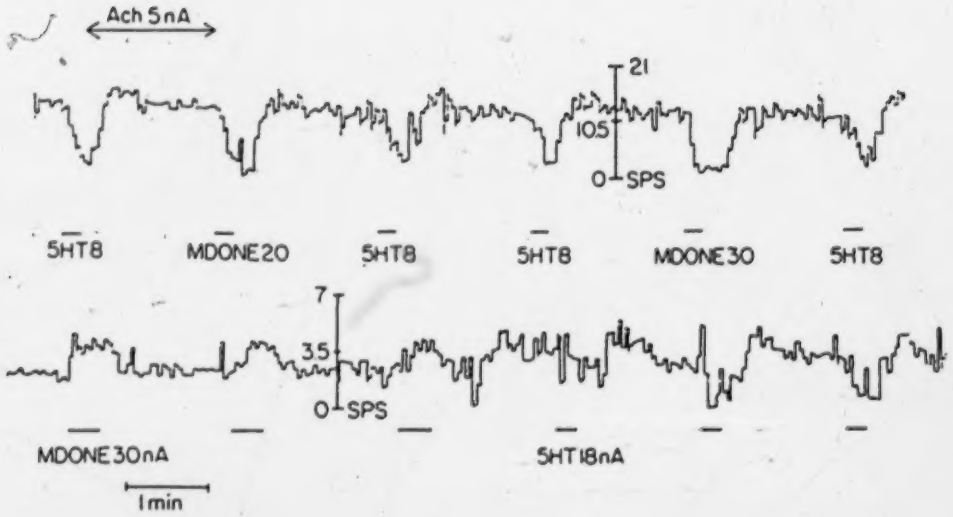

Fig. 5. Records of the firing of a hippocampal CA_1 cell when cell activity is maintained by acetylcholine (ACh) (upper trace) and when the cell firing is spontaneous (lower trace). The two traces are from the same cell. Upper trace: direct application of methadone (MDONE) and 5-HT suppresses the firing of this cell. Lower trace: in the absence of ACh the cell firing (at a lower rate) is not suppressed by MDONE but increased by about 50%. 5-HT still inhibits this cell under the same conditions.